THE PROGNOSTIC VALUE OF SINGLE FIBER ELECTROMYOGRAPHY OF THE ORBICULARIS OCULI MUSCLE IN PATIENTS WITH MYASTHENIA GRAVIS

Doctoral Dissertation

NAPOVEDNA VREDNOST MIKROELEKTROMIOGRAFIJE MIŠICE ORBICULARIS OCULI PRI BOLNIKIH Z MIASTENIJO GRAVIS

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The prognostic value of single fiber electromyography of the orbicularis oculi muscle in patients with myasthenia gravis

Napovedna vrednost mikroelektromiografije mišice orbicularis oculi pri bolnikih z miastenijo gravis

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ABSTRACT

Background
Myasthenia gravis (MG) is the most common autoimmune disease affecting the neuromuscular junction. The clinical course of MG is variable, ranging from remission in an early stage to an acute exacerbation and even death. The medical strategy for treatment may differ for patients with severe disease and it is therefore important to identify them as early as possible. At present there is no known prognostic factor that would predict the long-term clinical course in MG patients at the time of diagnosis.

Single fiber EMG (SFEMG) is considered the most sensitive diagnostic tool for the diagnosis of MG. The typical SFEMG findings in MG are increased jitter and/or impulse blocking in at least 10% of the examined motor end-plates. The sensitivity and specificity of the method depends on the muscle investigated and is highest for the orbicularis oculi muscle with a sensitivity and specificity range of 94-99% and 85-98% respectively.

The orbicularis oculi muscle is affected early in the disease clinical course in virtually all patients with MG. We noticed that many patients with MG with the same level of clinical disability significantly differ in respect of the extent of changes in SFEMG parameters of the orbicularis oculi muscle. The prognostic significance of these changes for the long-term clinical course of the disease has not been systematically studied.

The aim of our study is to determine if the extent of changes in single fiber EMG (SFEMG) of the orbicularis oculi muscle is predictive of the severity of the long-term clinical course of MG.

Hypothesis
The extent of the initial SFEMG abnormalities of the orbicularis oculi muscle correlates with the severity of the later clinical course of MG.

Methods
This was a retrospective observational study on patients diagnosed with MG between January, 1 2003 and December, 31 2012 at the Departments of neurology of the University Medical Centers of Ljubljana and Maribor. We reviewed medical files of 232 MG patients who presented with mild disease symptoms (score I-II according to the MG Foundation of America Clinical Classification Scale (MGFA CCS)) and had a SFEMG of the orbicularis oculi muscle at the time of first presentation. We rated the severity of the of patients' clinical course by the worst MGFA CCS reached by a patient during the observation period. Patients were then divided into two outcome groups: group 0 (considered benign, with the worst MGFA CCS of I-III) and group 1 (considered severe, with the worst MGFA CCS of IV-V). The two outcome groups were then compared for SFEMG characteristics and other clinical parameters that could potentially influence the outcome using uni- and multivariate logistic regression analysis.

Cut-off values of SFEMG parameters predicting severe disease clinical course were estimated by calculating the g-means (defined as geometric mean of sensitivity and specificity). Sensitivity and specificity were calculated with leave-one-out cross-validation from separate univariate logistic regression models.
Results
During the observation period 39 patients (17%) developed severe disease exacerbations whereas 193 patients (83%) remained stable. Patients with severe disease exacerbation had a significant higher mean jitter value (p<0.0001), a greater degree of fibers with increased jitter (p<0.0001), and/or impulse blocking (p<0.0001) on SFEMG. The association between initial SFEMG characteristics and later clinical outcome was confirmed by logistic regression analysis. This association persisted also when we adjusted for the effect of other clinical characteristics that could potentially influence the outcome according to multivariate logistic regression analysis (namely age and the presence of generalized disease symptoms). The cut-off values of SFEMG parameters that predicted a severe clinical course were ≥68.61μs for mean jitter, ≥81.31% of individual motor end-plates with increased jitter, and ≥21.86% of individual motor end-plates with impulse blocking.

Conclusions
In our study the extent of the SFEMG abnormalities correlated with the severity of the later clinical course of MG. In the study population, severe disease exacerbations were observed in subjects with a mean jitter value greater than 68.61μs, at least 81.31% of motor end-plates with increased jitter, and/or at least 21.87% of motor end-plates with conduction block. On the other hand, patients with lower mean jitter value, lower percentage of motor end-plates with increased jitter, and/or conduction block had a more benign clinical course. The hypothesis of our study thus seems confirmed.
IZVLEČEK

Izhodišča
Miastenija gravis (MG) je najpogostejše pridobljeno avtoimunsko obolenje živčno-mišičnega stika za katero sta značilni nihajoča šibkost in utrudljivost skeletnih mišic. Klinični potek bolezni in s tem prizadetost bolnikov je različna; nekateri zgodaj v poteku bolezni spontano dosežejo popolno stabilno remisijo bolezni, drugi večkrat doživijo huda poslabšanja ali pa celo smrt zaradi bolezni. Bolniki, ki so visoko ogrozeni za huda poslabšanja MG, bi že zgodaj v poteku bolezni potrebovali intenzivnejše klinično spremljanje in imunosupresivno zdravljenje. Prognostični dejavniki, ki bi nam zgodaj v poteku bolezni pomagali pri identifikaciji takih posameznikov, zazenkrat niso poznani.

Mikroelektromiografija skeletnih mišic (MEMG) je najbolj občutljiv diagnostični test za postavitev diagnoze MG. MEMG najdbe, ki so značilne za postavitev diagnoze miastenije gravis, so povečana srednja vrednost drgeta in/ali bloki prevajanja na najmanj 10% testiranih motoričnih ploščic. Občutljivost in specifičnost testa sta odvisni od testirane skeletne mišice in sta najvišji za mišico orbicularis oculi. Za mišico *orbicularis oculi* se občutljivost testa giblje med 94-99%, specifičnost testa pa med 85-98%.

Mišica *orbicularis oculi* (OOc) je prizadeta zgodaj v poteku bolezni praktično pri vseh bolnikih z MG.

Opazili smo, da se veliko bolnikov z MG ob enaki stopnji klinične prizadetosti medsebojno zelo razlikuje v obsegu MEMG sprememb mišice *orbicularis oculi*. Prognostični pomen izraženosti MEMG sprememb v mišici orbicularis oculi ob postavitvi diagnoze MG za dolgoročni klinični potek bolezni pa do sedaj še ni bil raziskovan.

Namen naloge je ugotoviti ali obsežnost izraženosti MEMG sprememb v mišici orbicularis oculi ob postavitvi diagnoze MG omogoča identifikacijo bolnikov, pri katerih je pričakovati težji potek bolezni.

Hipoteza
Obsežnost sprememb MEMG parametrov OOc (srednje vrednosti drgeta, odstotek motoričnih ploščic s povečano srednjo vrednostjo drgeta in/ali bloki prevajanja) ob postavitvi diagnoze MG neodvisno napoveduje težo nadaljnega kliničnega poteka bolezni.

Višja srednja vrednost drgeta, višji odstotek motoričnih ploščic s povečano srednjo vrednostjo drgeta in/ali bloki prevajanja pri začetni mikroelektromiografiji OOc, so napovedni dejavniki za težji klinični potek MG.
Metode
Hipotezo doktorske naloge smo preizkusili v retrospektivni observacijski raziskavi. Analizirali smo razpoložljivo medicinsko dokumentacijo bolnikov, ki so bili diagnosticirani z diagnozo MG na Nevrološki kliniki UKC Ljubljana in Oddelku za nevrološke bolezni UKC Maribor med 1.1.2003 in 31.12.2012. V raziskavo smo zajeli le bolnike, ki so imeli ob postavitvi diagnoze le blago izraženo prizadetost zaradi osnovne bolezni in so ob postavitvi diagnoze opravili MEMG OOC.
Za vsakega bolnika posebej smo poleg vrednosti MEMG parametrov izmerjenih ob postavitvi diagnoze, zbrali še osnovne demografske podatke, podatke o izraženosti simptomov in znakov MG, prisotnosti protiteles za acetilholinske receptorje v serumu, prisotnosti timoma, pridruženih obolenjih ščitnice, pridruženih drugih avtoimunskih obolenjih, pridruženih drugih malignih obolenjih, pridruženih kroničnih obolenjih, opravljeni timektomiji in uporabi imunosupresivnih zdravil. Simptome in znake MG smo pri vsakem zabeleženem obisku ocenili s pomočjo lestvice MG Foundation of America Clinical Classification Scale (MGFA CCS).
Na osnovi največje dosežene klinične prizadetosti znotraj opazovanega obdobja (najvišje dosežene ocene glede na lestvico MGFA CCS), smo preiskovance razdelili v dve skupini: skupino 0 (benigni potek, najvišja ocena po lestvici MGFA CCS I-III) in skupino 1 (hud potek, najvišja ocena po lestvici MGFA CCS IV ali V). Obe skupini smo primerjali glede MEMG parametrov (srednja vrednost drgeta, odstotek vlaken s povečano povprečno vrednostjo drgeta, odstotek vlaken z izraženimi bloki prevajanja) in drugih kliničnih značilnosti, ki bi lahko potencialno vplivali na klinični potek bolezni.
Povezavo med zbranimi parametri začetne MEMG mišice OOC in drugimi kliničnimi parametri ter izidno skupino smo vrednotili z uni- in multivariatno logistično regresijo. S pomočjo geometrične sredine občutljivosti in specifičnosti smo določili mejne vrednosti posameznih MEMG parametrov, ki napovedujejo težji klinični potek MG. Občutljivost in specifičnost smo za vsak MEMG parameter posebej izračunali z metodo prečnega preverjanja z izločitvijo enega iz posameznih univariatnih modelov logistične regresije.

Rezultati
V raziskavo smo vključili 232 bolnikov. V opazovanem obdobju je 39 bolnikov (17%) doživelo huda poslabšanja MG (skupina 1), preostalih 193 bolnikov (83%) pa je ostalo klinično stabilnih (izidna skupina 0).
Primerjava obeh skupin bolnikov je pokazala, da so imeli bolniki, ki so doživeli huda poslabšanja osnovne bolezni (skupina 1) v primerjavi z ostalimi bolniki(skupina 0), statistično pomemben višje srednje vrednosti drgeta (p <0,0001), večji odstotek motoričnih ploščic s povečanim drgetom (p <0,0001) in/ali bloki prevajanja (p <0,0001) na začetni MEMG OOC. Statistično pomemben povezavo med vrednostjo posameznih parametrov začetne MEMG OOC in skupino (kliničnim potekom MG) smo potrdili z metodo logistične regresije. Povezava med MEMG parametri in skupino je ostala statistično pomembna tudi potem, ko smo te parametre vključili v multivariatni model skupaj z drugimi kliničnimi parametri, ki so bili signifikantno povezani s skupino v posameznih univariatnih modelih (t.j. starost ter prisotnost
generaliziranih simptomov in znakov bolezni). Mejne vrednosti parametrov MEMG O Oc, ki so ob nastopu bolezni napovedovale možnost težkih poslabšanj MG so bile: srednja vrednost drgeta ≥68.6 μs, ≥81.3% individualnih motoričnih ploščic s povečano srednjo vrednostjo drgeta in ≥21.8% individualnih motoričnih ploščic z bloki prevajanja.

Zaključek
V raziskavi smo potrdili hipotezo doktorske naloge. V opazovani populaciji so srednja vrednost drgeta ≥68.6μs, ≥81.3% individualnih motoričnih ploščic s povečano srednjo vrednostjo drgeta in/ali ≥21.8% individualnih motoričnih ploščic z bloki prevajanja na začetni MEMG mišice orbicularis oculi neodvisno od drugih kliničnih spremenljivk napovedovali pojav hudih poslabšanj MG; nižje srednje vrednosti drgeta, nižji odstotek individualnih motoričnih ploščic s povečano srednjo vrednostjo drgeta in/ali nižji odstotek individualnih motoričnih ploščic z bloki prevajanja, pa je bil povezan z bolj benignim potekom bolezni. Naši rezultati kažejo na to, da ima mikroelektromiografija mišice orbicularis oculi pri bolnikih z MG poleg diagnostične, tudi napovedno vrednost za dolgoročni potek bolezni.
To Bond
(17.6.2003-18.7.2016)...

...the best friend I ever had.
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LIST OF ABBREVIATIONS

AChR - acetylcholine receptors
AIRE - autoimmune regulator element
BAFF - B-cell activating factor
CMAP - compound muscle action potential
CNE - concentric needle electrode
ED - extensor digitorum muscle
EFNS – European Federation of Neurological Societies
EPP - endplate potential
GCs - lymphoid germinal centers
IQR - interquartile range
IVIG - Intravenous immunoglobulins
Kv1.4 - α subunit of the voltage-gated K⁺ channels
LRP4 - low-density lipoprotein receptor-related protein 4
MCD - mean difference between consecutive action potential latencies
MG - Myasthenia gravis
MGFA - Myasthenia Gravis Foundation of America
MuSK - muscle specific kinase
NMJ - neuromuscular junction
OOc - orbicularis oculi muscle
PE - plasma exchange
QMGS - quantitative myasthenia gravis score
RNS - Repetitive nerve stimulation
RyR - Ryanodine receptor
1. INTRODUCTION

MYASTHENIA GRAVIS (MG)

Myasthenia gravis (MG) is the most common autoimmune disorder of the neuromuscular junction (NMJ)(1). It is caused by antibodies against proteins of the postsynaptic membrane of which three have been identified - namely acetylcholine receptor (AChR), muscle specific kinase (MuSK) and low-density lipoprotein receptor-related protein 4 (LRP4) (1-3).

MG is characterized by painless fluctuating skeletal muscle weakness that worsens with activity and improves with rest. Weakness typically varies from day to day and perhaps from hour to hour, but is generally worse later in the day. It can be focal or generalized, and usually affects ocular, bulbar and proximal extremity muscles. Respiratory muscle weakness develops only rarely, but can be life-threatening (1,4).

Thomas Willis was probably the first to describe a myasthenic patient in his book »De Anima Brutorum« in 1672. He described a »prudent and honest woman« suffering from fluctuating weakness in the arms and legs but also from compromised articulation (5,6). However, prior to the introduction in 1934 of cholinesterase inhibitors for diagnosis and management, the few people recognized to have MG, were mainly those with severe weakness who died within 1 or 2 years. Between 1915 and 1934 70% of the recognized MG patients died of respiratory failure or pneumonia. After 1934, when anticholineseterase compounds improved diagnosis and management, mortality declined to 30% by 1955. A further dramatic decrease in mortality happened in the next decades following the introduction of thymectomy, immunosuppressive treatment, immunomodulatory treatment and improvement in the intensive care (7). With the current treatment possibilities, MG has become one
of the most treatable neurologic disorders (8). As a result of optimal treatment, the disease can be managed satisfactorily in most instances with good long–term prognosis and normal life expectancy (9-11).

EPIDEMIOLOGY OF MG

MG occurs in all races, both genders and all ages (7,11). The disease prevalence has increased over time with recent estimates approaching 150-300 per 1000 000 persons with slight variation between populations (12-14). This increased prevalence is most likely to be due to improved diagnosis and treatment of MG, and increasing longevity of the population in general (15). Since its prevalence is less than 500 per 1000 000 persons, MG is considered a rare disease according to the European union definition (2).

The incidence of MG has also increased over time varying widely from 1.7 to 24.9 per 1000 000, depending on the location of the study (15-17). The occurrence of MG is influenced by sex and age: women are affected nearly three times more often than men during early adulthood (age<40 years), whereas incidence is roughly equal during puberty and after the age of 40 years (7,15). After 50 years of age, incidence is higher in men (7, 15). In recent years, some studies have reported a steady increase in MG incidence for both sexes with a peak in the 6th and 7th decade (11, 13, 18, 19), whereas other studies (mainly from Scandinavia, UK and Italy) have reported a bimodal age distribution pattern for women, with a first peak in the 2nd and 3rd decade and a second peak in the 6th and 7th decade (14). Childhood MG is uncommon in Europe and North America, comprising 10-15% of MG cases (15, 20), but is much more common in Asian countries, where up to 50%
of patients have disease under the age of 15 years, many with purely ocular manifestations (15, 21).

**CLINICAL PRESENTATION OF MG**

The clinical hallmark of MG is fatigable weakness, usually involving specific susceptible muscle groups. Patients often note that their weakness fluctuates from day to day or even from hour to hour, worsens with activity and improves with rest. Patients can have varying degrees of ptosis, diplopia, dysarthria, dysphagia, dyspnea, facial weakness or fatigable limb or axial weakness (15).

Asymmetric weakness of ocular muscles, manifesting as fluctuating ptosis and/or diplopia, is the most common initial presentation of MG, occurring in approximately 70% of patients, but virtually all patients will have ocular muscle involvement within two years of the disease onset (3, 22).

Initial involvement of other than extra-ocular muscles occurs much more rarely: bulbar weakness with painless dysphagia, dysarthria or chewing difficulties, is the initial symptom in up to 15% of patients, approximately 10% of MG patients present with proximal limb muscle weakness and in less than 5% of patients MG initially manifests with respiratory muscle weakness (1).

MG remains purely ocular in 15% of cases. Disease generalization usually occurs within two years from symptoms onset (15). Only 15% patients progress from ocular MG to generalized MG after 2 years of disease duration (3, 23). When MG generalizes, the symptoms of muscle weakness typically progress in a craniocaudal direction: from ocular to facial to lower bulbar to truncal, and finally to limb muscles (1).
The course of MG is variable. Many patients experience intermittent worsening of symptoms triggered by infections, emotional stress, surgeries or medications, particularly during the first year of the disease (15). Progression to maximal severity typically occurs within the first 2 years of onset, when despite modern treatments, at least 20% of patients will experience a myasthenic crisis, defined as weakness necessitating intubation and mechanical ventilation (7, 15). Spontaneous long-lasting remissions are uncommon, but have been reported in 10-20% of patients (7, 15).

**PATHOGENESIS OF MG**

MG serves as a prototypical antibody mediated disease because the physiological function, antigenic targets, and immune responses to autoantibodies at the postsynaptic membrane of the NMJ are well understood (12).

**Normal function of the NMJ**

The normal NMJ has three basic components (figure 1):

- the presynaptic motor nerve terminal (the axon terminal), where Ach and agrin are synthesized, stored and released
- the synaptic cleft
- the postsynaptic muscle membrane, which contains the AChR, the enzyme acetylcholinesterase, the LRP4 and the MuSK (12,15).
FIGURE 1. Normal function of the NMJ with major components implicated in MG (12)

Action potential at the presynaptic nerve terminal causes opening of voltage-dependent Ca^{2+} channels, triggering release of acetylcholine and agrin into the synaptic cleft. Acetylcholine binds to the AChR, which promote sodium channel opening, which in turn triggers muscle contraction. Agrin binds to the complex formed by LRP4 and MuSK, causing AChR clustering, which is required for maintenance of the postsynaptic structures of the NMJ. AChR= acetylcholine receptors, LRP4= lipoprotein receptor-related protein 4, MuSK= muscle specific kinase, RyR= ryanodine receptor antibodies, Kv1.4= voltage-gated K^{+} channels, ColQ= collagen Q, NMJ= neuromuscular junction, MG= myasthenia gravis.

In normal neuromuscular transmission, the action potential at the presynaptic nerve terminal causes opening of voltage dependent Ca^{2+} channels, triggering release of acetylcholine and agrin into the synaptic cleft. Acetylcholine binds to the AChR, which promote sodium channel opening, causing a local depolarization- the endplate potential (EPP), which in turn triggers muscle contraction. The EPP in normal NMJ is much larger than the threshold for generation of a muscle fiber action potential; this difference has been defined as the safety factor of neuromuscular transmission. The action of acetylcholine on the postsynaptic membrane is terminated by acetylcholinesterase (12, 15).
Agrin, released together with acetylcholine from the presynaptic nerve terminal, is a heparan sulfate proteoglycan, which binds to the complex formed by LRP4 and MuSK, causing AChR clustering, required for maintenance of the postsynaptic structures of the NMJ (12, 15).

The NMJ in MG

In MG, different autoantibodies target specific proteins of the postsynaptic muscle endplate causing loss of functional AChRs. As a consequence, the EPP amplitudes fall below the threshold required for muscle fiber action potential generation during repetitive nerve depolarization, resulting in neuromuscular transmission failure (12, 15).

In the majority of MG cases, these pathogenic autoantibodies can be detected in patients’ serum.

The most common antibodies detected in MG are:

- AChR antibodies
- Clustered AchR antibodies
- MuSK antibodies
- LRP4 antibodies

Patients without detectable antibodies against any of the above mentioned antigens are referred to as seronegative (12, 24, 25).

Some patients with MG also have antibodies against cytoplasmic muscle proteins and/or other endplate proteins; although these antibodies are not pathogenic, they can serve as disease markers (12, 26-29).

The most relevant features of each antibody are summarized in the following discussion.
AChR antibodies

**Prevalence.** AChR antibodies can be detected with routine assays in 70% of all patients with MG (12,24, 30-32).

**Pathophysiological role.** AChR antibodies are predominantly of the isotype IgG1 and IgG3 (33,34), and exert their pathogenic role by binding to and resulting in the loss of functional AChR by 3 primary mechanisms: focal lysis of the endplate membrane via activation of complement; crosslinking of adjacent receptors promoting internalization and degradation; and direct blockade of the acetylcholine binding site (Fig.2) (33,35-38).

**FIGURE 2.** Three mechanisms of endplate pathology in AChR antibody positive MG. (1) Antibodies bind to AchR and activate complement leading to focal endplate lysis; (2) antibodies crosslink adjacent AChR leading to internalization and degradation; (3) antibodies directly bind to acetylcholine binding site blocking access to acetylcholine (33).

In anti-AChR-positive MG, the production of autoantibodies by pathogenic B cells is T cell-dependent(33, 39,40). CD4+T helper (Th) and T regulatory (Treg) cells recognize AChR epitopes in the context of major histocompatibility complex class II and stimulate B cells to proliferate and differentiate into plasma cells (33). Patients with anti-AChR-positive MG may be further subdivided into those with and without thymic pathology. Approximately 70% of patients with MG with anti-AChR antibodies have thymic follicular hyperplasia, approximately 10% have thymomas, and the remaining have a histologically normal or atrophic thymus gland (33, 41-43). The alternations of the immune system that occur with thymic hyperplasia
vs. thymoma are quite distinct (33). In patients with thymic hyperplasia, there is evidence that the thymus is the primary site of immune sensitization to the AChR and may play a role in perpetuating the disease (33, 44, 45). Thymic follicular hyperplasia usually occurs in early onset MG and is characterized by the development of lymphoid germinal centers (GCs) containing a large number of B cells (33). The formation of these ectopic GCs may be triggered by a viral infection or other source of inflammation (33, 46), but this has not been clearly demonstrated (33). GC formation is associated with an overexpression of proinflammatory cytokines and a chain of events including enhanced α-AChR expression in thymic epithelial cells (TECs), recruitment of peripheral B cells, a dysfunction in Tregs, and, eventually, intrathymic autoantibody production (33, 41). Thus, the thymic GC environment in MG promotes the survival and differentiation of AChR-specific B cells and the production of antibodies (33, 45, 47).

Tumors originating from TECs are called thymomas, and are found in about 10-15% of patients with MG (33, 43, 48), usually in patients with generalized MG with disease onset when they are >40 years of age (33). These tumors commonly express self-antigens such as the AchR and the large muscle protein, titin. Naive effector T cells with AChR or titin reactivity may escape negative selection due to the abnormal thymic microenvironment created by the thymoma (33, 43). Alternatively, autoimmunization may actually occur in the thymus owing to expression of AChR by the tumor and the derangement of normal immune regulation due to a deficiency of AIRE (autoimmune regulator element; a transcriptional activator expressed by medullary TECs) (33, 49). In either case, there is export of potentially autoreactive or »primed« T cells that have the ability to stimulate a B-cell response in the periphery. Not surprisingly, the autoimmune response may be self-sustaining in these patients, persisting even after the tumor is removed (33).

**Testing.** A radioimmunoprecipitation assay based on a mixture of solubilized embryonic and adult AchRs is the most rigorously validated test for AChR antibodies, and is the most reliable among the validated AChR antibody tests. These testing kits are commercially available. Positive test results have a near 100% specificity for MG in symptomatic
individuals (12, 24, 30-32). Although ELISA and immunofluorescence assays have been developed as non-radioactive alternatives with good sensitivity and specificity, they are inferior to radioimmunoprecipitation (50) and have not been widely adopted in clinical practice (12,50).

The radioimmunoassay is recommended as the first line test because it has been used successfully in clinical practice; moreover, it not only detects the presence of AChR antibodies but can also quantify their levels (12). This might have implication in clinical practice, since fluctuations in AChR antibody concentration in an individual patient have been reported to correlate with the severity of muscle weakness and to predict exacerbations. Such correlations remain to be convincingly demonstrated, but repeated testing for antibodies can influence therapeutic decisions: an increase in antibody concentration is thought to indicate exacerbation of MG, whereas a stable or decreasing concentration could indicate stable disease (12, 51). Interestingly, total AChR antibody concentration does not correlate with symptom severity when patients are compared (12, 24, 30-32).

**Clustered AChR antibodies (low-affinity AChR antibodies)**

*Prevalence.* Clustered AchR antibodies are detected with cell-based assays in 5-10% of patients with MG (52). These patients are seronegative for AChR antibodies using the conventional radioimmunoprecipitation assay (52-54).

*Pathophysiological role.* Clustered AchR antibodies belong to the same IgG subclass as AChR antibodies that are detected with the conventional radioimmunoprecipitation assay, thus indicating a common etiology and pathogenesis, including the presence of thymic hyperplasia (12).

*Testing.* Cell based assay have high sensitivity but are not yet commercially available and standardized. If possible, cell-based testing should be performed when MG is suspected but the patient is negative for anti-AChR and anti MuSK antibodies (12).
MuSK antibodies

**Prevalence.** MuSK antibodies can be detected in 1-10% of patients with MG (12). These antibodies are present in patients from the Mediterranean area more often than those from Northern Europe, possibly owing to a combination of genetic and environmental factors (12, 24, 30-32).

**Pathophysiological role.** MuSK antibodies are of the IgG4 isotype and are directly pathogenic (12). Most notably, MuSK antibodies reduce the postsynaptic density of AChR and impair their alignment on the postsynaptic membrane of the NMJ by perturbation of the agrin-MuSK-LRP4 pathway (33, 55, 56). The trigger for the production of anti-MuSK antibodies is not known, and the role of T cells in the production of pathogenic anti-MuSK antibodies is incompletely investigated (33). Thymic pathology does not commonly occur in association with the disease (57). B-cell immunopathology in MuSK MG includes elevated B-cell activating factor (BAFF) and lower frequencies of interleukin-10-producing regulatory B cells (58).

**Testing.** Immunoprecipitation with radioimmunoassays is the standard technique for MuSK antibody detection, and ELISA tests are also available, although neither are as sensitive as cell-based assays (59, 60): a large screening study showed that cell-based assay could detect MuSK antibodies in 13% of MG serum samples that had been classified as seronegative using radioimmunoassay or ELISA (26). The specificity of the cell-based assay is estimated to be 97-98% (12).

In patients with MuSK-MG, concentrations of anti-MuSK antibody tend to correlate with disease severity, and changes in antibody concentration over time can reflect disease activity (60).

MuSK-MG and AChR-MG are distinct disease entities and rarely occur in the same patient. Nevertheless, a few single-patient reports have demonstrated the existence of both AChR and MuSK antibodies in the same patient, particularly when the most sensitive antibody detection techniques are used (12, 61).
LRP4 antibodies

**Prevalence.** LRP4 antibodies are detected in 1-5% of patients with MG of any type (62,63), and in 7-33% of MG patients without AChR and MuSK antibodies (63,64). The likelihood of detecting LRP4 antibodies in MG depends on the assay and the examined population (12).

**Pathophysiological role.** LRP4 antibodies belong to the complement binding IgG1 subclass (65). In mice models, LRP4 antibodies are directly pathogenic and induce muscular weakness through disruption of the interaction between LRP4 and agrin, causing impairment of AChR-mediated neuromuscular transmission (12, 66). The disease in LRP4-antibody mouse model was similar to that seen in mice immunized with AchRs, and to muscle weakness seen in human disease (12). The fact that LRP4 antibodies are primarily reported in MG patients without AChR or MuSK antibodies suggests that LRP4-MG is a distinct disorder (62). However, LRP4 antibodies have recently been detected in several patients with either AChR or MuSK antibodies, in patients with other autoimmune disorders, and in patients with amyotrophic lateral sclerosis (12, 62, 63, 67). The presence of LRP4 antibodies is associated with milder MG symptoms, and LRP4-MG can manifest purely as ocular MG (12, 62, 63, 67).

**Testing.** No commercial tests for LRP4 antibody are available. Optimal testing conditions and thresholds for sensitivity and specificity in a clinical setting have not yet been defined (12).

Other antibodies

**Agrin antibodies.** Agrin antibodies can be detected in a minority of patients with MG, either with or without antibodies against AChR, MuSK or LRP4 (68, 69). Agrin antibodies have been detected only in patients with MG, suggesting that these antibodies are specific to the disease (12). However, to date, no directly pathogenic effect of agrin antibodies has been established in vivo, although in vitro such antibodies inhibit MuSK phosphorylation and AChR clustering (68-70).
**Titin antibodies.** Titin is the third most abundant protein in skeletal muscle and the largest protein in the body. It is responsible for the passive elasticity of skeletal muscles (12). Titin antibodies are present in 20-30% of MG patients with AChR antibodies (71). Their presence indicates a more severe form of MG with mild myopathy and a need for immunosuppressive treatment (12, 26). Moreover, titin antibodies are a sensitive marker of thymoma in patients with MG onset before the 50 years of age (12, 26, 71). A commercial kit is available for titin antibody testing (12).

**Kv1.4 antibodies.** Kv1.4 antibodies are antibodies against the α subunit of the voltage-gated K⁺ channels (12) which are located mainly in the brain, peripheral nerves, skeletal and heart muscles (11).

Kv1.4 antibodies are detected in 10-20% of patients with MG and seem to cross-react with voltage-gated K⁺ channels in the heart muscle (29,72). In a Japanese patients' cohort, Kv1.4 antibodies were associated with severe MG and heart complications (73), but these findings were not confirmed in European patients (72), suggesting different clinical phenotypes between the two populations (12). Kv1.4 antibodies can be detected by an immunoprecipitation assay using 35S-labeled rhabdomyosarcoma cellular extract as the antigen source (11, 73). Their presence is usually associated with a more severe disease and a need for a more active treatment (12).

**Ryanodine receptor antibodies.** Ryanodine receptor (RyR) is the Ca²⁺ channel on the sarcoplasmic reticulum. It opens upon depolarization of the sarcolemma and participates in muscle contraction through release of calcium from the sarcolemma into the cytoplasm (12, 27). RyR antibodies are present in 70% of AChR-MG patients with thymoma and in 14% of patients with late-onset AChR-MG (10, 74). Their presence indicates severe MG, though the pathogenic role of RyR antibodies has not been established (12, 27).
**Collagen Q antibodies.** Collagen Q is a protein that concentrates and anchors acetylcholinesterase at the synaptic membrane of the NMJ. It is found only at the NMJ (12). Collagen Q antibodies were recently detected in the serum of 12 of 415 (3%) patients with MG. In 7 of these patients, no other antibodies were found (75). However, anti-collagen Q antibodies have also been detected in healthy controls, and any diagnostic or pathogenic significance of these antigens remains to be proven (12).

**Cortactin antibodies.** Cortactin is a cytosolic actin-binding protein in the skeletal muscle that promotes actin assembly. Moreover, it has a role as a signaling protein involved in AChR clustering mediated by the agrin-MuSK complex (12, 76). Cortactin antibodies were recently detected in 20% of MG patients without AChR or MuSK antibodies, but also in 5% of MG patients with AChR antibodies, healthy controls, and 13% of patients with various autoimmune disorders, including myositis (26). Any relevance to MG remains to be proven (12).
DIAGNOSIS OF MG

The diagnosis of MG is primarily based on characteristic clinical history and examination findings that should be objectively confirmed using specific diagnostic tests.

Clinical diagnosis

The clinical hallmark of MG is the presence of fatigable muscle weakness. It is useful to distinguish fatigable muscle weakness from general fatigue or exhaustion. Patients with fluctuating fatigable muscle weakness due to MG will describe weakness in a specific group of muscles that is brought on by activity and improves with rest. In contrast, patients with general fatigue or exhaustion due to any number of causes will typically report all-over weakness, tiredness, or lack of energy (22). Patients with MG may have symptoms and signs only after exertion or at the end of the day. This may result in little detectable objective weakness at the time of examination, often delaying the diagnosis (22). Maneuvers that fatigue specific muscle groups can be very useful in eliciting signs of weakness in patients with MG because patients with generalized fatigue or malaise do not typically display true muscle weakness with these maneuvers (table 1) (22).

Table 1: Fatiguing Maneuvers in suspected MG (22).

<table>
<thead>
<tr>
<th>Clinical Fatiguing Maneuver</th>
<th>Manifestation in Symptomatic MG</th>
</tr>
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<tbody>
<tr>
<td>Sustained upgaze (30 to 60s)</td>
<td>Enhances ptosis and elicits medial rectus weakness</td>
</tr>
<tr>
<td>Sustained abduction of the arms (120s)</td>
<td>Patient can no longer hold arms up, or weakness becomes apparent with subsequent manual testing</td>
</tr>
<tr>
<td>Sustained elevation of leg while lying supine (90s)</td>
<td>Patient can no longer hold leg up, or weakness becomes apparent with subsequent manual testing</td>
</tr>
<tr>
<td>Repeated arising from chair without use of arms (up to 20)</td>
<td>Fatigues after several attempts</td>
</tr>
<tr>
<td>Counting aloud (1 to 50)</td>
<td>Enhances dysarthria</td>
</tr>
</tbody>
</table>
Specific diagnostic tests in MG

The major tools used to confirm the diagnosis of MG could be divided into five main groups: clinical, pharmacologic, electrophysiologic, immunologic and imaging.

Clinical tests

Ice-pack test. The test is applicable only in patients presenting with ptosis. Ice between 0 and 4°C is placed over the closed eyelids of both eyes for 2 minutes (figure 3). Photographs before and after can be taken to objectively record the results of the test. The test is considered positive for the diagnosis of MG when the palpebral fissure widens by more than 2mm (ptosis improves by 2mm or more) from baseline. More positive responses are seen in MG when ptosis is partial rather than complete. It has a sensitivity of 90-95% and a specificity of 100% (77).

FIGURE 3. Ice pack test (77).

Obviously right ptosis (A) before placing ice over both eyes for 2 minutes (B). The ptosis has resolved (C).
Pharmacologic tests

Edrophonium chloride test. By inhibiting the normal action of acetylcholinesterase, edrophonium chloride and other acetylcholinesterase inhibitors allow acetylcholine molecules to diffuse more widely throughout the synaptic cleft and to interact with AChRs sequentially, increasing the amplitude and duration of the EPP (15). Edrophonium chloride has a rapid onset of action (30 seconds) and a short duration of effect (5-10 minutes) (78). The test consists of administering edrophonium intravenously and observing for an improvement in muscle strength. Most commonly, a test dose of up to 2mg is given followed by subsequent doses of 3mg to 8mg until there is a positive response or a total of 10mg is given. The patient is observed for 90 seconds in between doses and for 3 to 5 minutes after the full of 10mg dose is administrated (78). The test can be objectively and reliably interpreted in only a few specific situations. The most important consideration in performance of the edrophonium test is the end point to be used. Only unequivocal improvement in strength of a sentinel muscle should be accepted as a positive result. For this reason, resolution of eyelid ptosis and improvement in strength of a single paretic extraocular muscle have been advocated as the only truly valid end points because observed function in these muscles is largely independent of fluctuating effort (78). Side effect from edrophonium include salivation, sweating, nausea, stomach cramping, and muscle fasciculations. Hypotension and bradycardia are infrequent and generally resolve with rest in the supine position, but atropine (0.4 to 2mg i.v.) should be available in the event that bradycardia persists (78). Published reports indicate that the sensitivity of the edrophonium test in the diagnosis of MG ranges from 71.5% to 95% for generalized MG (79). The specificity of the test is not clear, but a positive response to edrophonium has been reported in a variety of conditions other than MG, including other disorders of the NMJ, such as Lambert-Eaton myasthenic syndrome and botulism, as well as in patients with motor neuron disease (79). Non responsiveness as well as hypersensitivity to cholinesterase inhibitors has been observed in patients with MG who have anti-MuSK antibodies (80).
Edrophonium chloride was previously an integrated part of the diagnostic procedure, but has recently been phased out in many clinics as the drug is not easily available (81).

**Neostigmine bromide test** (92). If edrophonium is not available, neostigmine bromide, a longer-acting cholinesterase inhibitor, can be given subcutaneously at a dose of 1-2mg (1mg/50kg body weight). Before neostigmine bromide, a dose of atropine should be given subcutaneously, which functions both as an anticholinergic drug and a placebo, immediately improving muscle strength. The use of atropine is advisable in case of predominant limb muscle weakness or diplopia without visible eye muscles paresis, which may occur in fatigue syndromes. The testing protocol and interpretation of the neostigmine bromide test is summarized in box 1 (82).

**Box 1: Neostigmine bromide test protocol (82).**

- Prepare the patient by telling her/him that she/he will get one or two injections, depending on the effect of the first
- Test target signs
- Administer 0.5mg atropine subcutaneously
- Test target signs after 10 minutes
- Administer 1-2mg (1mg/50kg body weight) neostigmine bromide i.m.
- Test target signs after 10, 20, 30 minutes and 2 hours

- **Maximal effect to be expected 10-20 minutes after the second injection!**
- **Effect of the first injection should be negative!**
- **Symptoms should reappear 1-2 hours after the second injection**
Electrophysiologic tests

Repetitive nerve stimulation (RNS) (83). RNS is the most commonly used electrophysiologic test of neuromuscular transmission (78). In this technique, a peripheral nerve is stimulated supramaximally and the compound muscle action potential (CMAP) is recorded. At low rates of stimulation (2Hz to 5Hz) RNS serves to stress diseased motor endplates by depleting the store of readily releasable acetylcholine, causing lowering of the EPP below the threshold for muscle fiber activation. As the EPP drops below threshold for muscle fiber activation in an increasing number of end plates, the number of muscle fibers contributing to the CMAP declines and the resulting CMAP is reduced in amplitude and area (decremental response) (figure 4). In a patient with MG, the typical pattern seen with RNS at 2 to 3Hz, is a progressive decrement of at least 10% from the second through the fourth or fifth response to a train of stimuli with some return toward the initial CMAP size during the subsequent stimulation- the so called »U-shaped pattern« (figure 4).

FIGURE 4. Repetitive nerve stimulation tracings (83).

Repetitive nerve stimulation tracings from a normal control subject (A) and a patient with MG (B) illustrating a classic »U shaped« response (a progressive decrement of at least 10% from the second through the fourth or fifth response to a train of 2 to 5 Hz stimulation with some return toward the initial CMAP size during the subsequent stimulation).

Although a seemingly simple test, careful attention to proper technique is important to avoid erroneous RNS results. The sensitivity of RNS for diagnosing MG ranges from 53% to 100%
for generalized MG, and 10% to 17% for ocular MG. In generalized MG, RNS studies are abnormal in approximately 60% of patients when a hand and a shoulder muscle are tested. RNS is more likely to be abnormal in a proximal or facial muscle in patients with MG and is more likely to be abnormal in clinically weak muscles. To obtain the maximal diagnostic yield, multiple muscles should be tested, particularly clinically weak muscles. Cholinesterase inhibitors could reverse the decremental RNS response in MG and thus mask minimal or mild abnormalities of neuromuscular transmission. Therefore, in order to avoid false negative results, anticholinesterase medications should be withheld 12-72 hours prior to testing, if this can be done safely. If RNS is normal and a high suspicion for MG exists, single fiber electromyography of at least one symptomatic muscle should be performed.

**Single fiber electromyography (SFEMG)** (83). SFEMG is a selective recording technique in which a specially constructed concentric needle is used to identify and record action potentials from individual muscle fibers. SFEMG is the most sensitive test for detection of a defect in neuromuscular transmission. Its sensitivity allows for demonstration of abnormalities in clinically unaffected muscles. When the motor nerve is stimulated or activated voluntarily, the latency from nerve activation to muscle action potential varies from discharge to discharge. This variation is the neuromuscular jitter and is produced by fluctuations in the time it takes for the EPP at the NMJ to reach the threshold for action potential generation. These fluctuations are in turn due to the normally varying amount of ACh released from the nerve terminal after a nerve impulse. A small amount of jitter is seen in normal muscles due to this phenomenon. An increase in the magnitude of this jitter is the most sensitive electrophysiological sign of a defect in neuromuscular transmission. When the defect in neuromuscular transmission is more severe, some nerve impulses fail to elicit action potentials and SFEMG recordings demonstrate intermittent impulse blocking. (figure 5).

SFEMG studies can be performed during either mild voluntary activation of the muscle under study or with axonal microstimulation. Jitter measurements performed during voluntary
activation of the muscle are less subject to technical problems but are more dependent on patient cooperation. As the patient minimally contracts the muscle under study, the examiner positions the recording electrode to record two or more time-locked action potentials. A constant recording position is maintained until at least 50 discharges are recorded. At least 20 potential pairs from different areas in the muscle should be sampled, taking care not to measure the same pair of potentials more than once.

**FIGURE 5. SFEMG with voluntary activation technique** (83)

The SFEMG needle is inserted into voluntarily activated muscle and is positioned so that the recordings are obtained from two or more single muscle fibers belonging to the same motor unit. One single muscle fiber action potential serves as a time reference and the interpotential interval (IPI) is measured after consecutive discharges between the reference potential and the subsequent time-locked potentials. In disorder of the neuromuscular junction, there may be marked variability of the IPI (abnormal jitter). If severe, neuromuscular transmission failure may occur in which the EPP amplitude fails to reach the threshold for action potential generation. This is demonstrated here by the absence of the second recorded fiber pair when the EPP in the second muscle fiber (EPP₂) is subthreshold (dotted lines and arrows).
Jitter is measured as the variation in the time interval between the two action potentials in the pair (interpotential interval) and represents the combined jitter in two end plates. In jitter studies performed with axonal microstimulation, action potentials from single muscle fibers are recorded during stimulation of motor nerve fibers with a monopolar needle inserted near the nerve. The stimulus to response interval is determinated for a series of 50 to 100 stimulations, and the variability of these intervals is a measure of the jitter in a single end plate (figure 6). This technique is prone to several pitfalls and misinterpretations but is useful when patients cannot sustain muscle contraction. Jitter is calculated as the mean difference between consecutive interpotential intervals (or stimulus response intervals). The mean jitter of all fiber pairs or end plates, the percentage with normal jitter, abnormal jitter, and impulse blocking are calculated and reported for each muscle tested. A study is abnormal if the mean jitter of all fiber pairs (or end plates) exceeds the upper limit of normal for that muscle, or if more than 10% of pairs or end plates have jitter that exceeds the upper limit of normal in that muscle.

**FIGURE 6. Normal jitter and abnormal jitter (83).**

An example of normal jitter (A); 40 consecutive discharges are superimposed. Abnormal jitter (B); 50 consecutive discharges are superimposed.
Reference values for jitter during voluntary activation have been determined for several muscles in a multicenter collaborative study (83,84). Normal jitter values for axonal microstimulation have been determined for some muscles. For other muscles, the normal values for stimulated jitter can be calculated by dividing the values for voluntary activated jitter by 1.4. SFEMG demonstrates increased jitter in virtually all patients with MG if appropriate muscles are tested. Jitter is greatest in weak muscles but is usually increased even in muscles with normal strength. Facial muscles are often more abnormal than limb muscles in most MG patients, and it may be necessary to test several muscles to demonstrate abnormal jitter in patients with mild or purely ocular weakness. The finding of normal jitter in a clinically weak muscle essentially rules-out a defect in neuromuscular transmission as a cause of weakness. It is important to understand that the enhanced sensitivity of SFEMG comes at the price of reduced specificity. Jitter may be increased in primary nerve or even muscle disease, and these disorders must be excluded by the appropriate electrophysiological and clinical examinations before concluding that the patient has MG.
**Immunologic and other serologic tests**

**AChR antibodies.** Antibodies that react with AChR proteins are generally regarded as specific serologic markers for acquired MG. Serum AChR-binding radioimmunoprecipitation assay is the most widely used diagnostic test for MG and should always be undertaken when MG is suspected (15, 85). Further aspects of the AChR-antibodies and the methods of testing are discussed in the section »PATHOGENESIS of MG«.

**MuSK antibodies.** Approximately 40% of patients with anti-AChR negative generalized MG have been found to have IgG anti-MuSK antibodies in their serum (86). MuSK antibodies are typically not found in anti-AChR positive MG or in ocular MG, although a few case reports of patients with ocular MG and anti-MuSK antibodies have been published (86). Serum anti-MuSK antibody testing should be undertaken in all patients negative for AChR-antibodies when MG is suspected (15, 85). See the section »PATHOGENESIS OF MG« for more details.

**Striational antibodies.** Antibodies against titin, ryanodine and Kv1.4 could be detected in a subset of AChR- antibody positive MG patients. Their presence in the serum of patients younger than 50 years is considered a sensitive marker for thymoma, whereas in patients older than 50 years, the presence of striational antibodies indicates severe MG with need for long-term immunosuppression and no response to thymectomy (24). Serum striational antibody testing should be thus considered in all AChR-antibody positive MG patients (12). More details are discussed in section »PATHOGENESIS OF MG«.

**Other serologic tests.** Because MG often coexists with other autoimmune disorders, particularly thyroid disease (24, 87), baseline testing of thyroid function should be obtained at the time of MG diagnosis (85), and other autoimmune serologics should be considered if clinically indicated (78).
Imaging

**Chest CT or MRI.** All patients with suspected MG, irrespective of weakness distribution (ocular/generalized) or serology status, should undergo thymus imaging to exclude the presence of a thymoma. The modality (CT or MRI) should be decided locally (84). Iodinated contrast agents should be used with caution because they might exacerbate myasthenic weakness (78).

**Brain MRI.** In seronegative patients, with normal neurophysiological results and absent or weak response to pharmacologic testing, other diagnoses must be considered (85). This situation is most often encountered in patients with purely ocular symptoms and signs, and in all such cases it is wise to perform brain MRI. The literature abounds with cases of intracranial structural disease initially masquerading as »seronegative MG«, usually involving the brainstem; III, IV or VI intracranial nerves; or orbit (88).
DIAGNOSTIC APPROACH

All patients with suspected MG should undergo testing for anti-AChR antibodies and if negative additional anti-MuSK antibody testing should be done (85). The detection of serum anti-AChR or anti-MuSK antibodies in a patient with appropriate clinical presentation essentially confirms the diagnosis of MG, and obviates the need for further testing (78). Antibody testing should be performed on nonimmununsuppressed patients and should be repeated after six months if initially negative as 15.2% of initially seronegative patients may become positive (89).

In practice, bedside (clinical and/or pharmacological testing) and electrophysiological tests are commonly done concurrently with antibody testing because the results of the later are usually delayed (78).

In patients with confirmed MG, chest CT or MRI and additional thyroid function tests should be obtained.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of MG is given in table 2. In general, alternative diagnosis to be considered include other disorders of the NMJ, motor neuron disease, primary muscle diseases (particularly those that affect ocular and pharyngeal muscles), and CNS lesions affecting the brainstem nuclei. Disorders such as chronic fatigue syndrome and certain mood disorders may also be considered, but symptoms in these cases usually consist of generalized exhaustion, malaise, apathy, and somnolence rather than true fatigable muscle weakness (15, 78).
**Table 2: Differential diagnosis of MG (15,78)**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Points of differentiation</th>
</tr>
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<tbody>
<tr>
<td>Lambert-Eaton myasthenic syndrome</td>
<td>Relative sparing of ocular muscles, hyporeflexia, autonomic features (dry mouth, impotence, postural hypotension)</td>
</tr>
<tr>
<td>Congenital myasthenic syndromes</td>
<td>Seronegative, onset in infancy or childhood; no response to immunomodulatory therapy</td>
</tr>
<tr>
<td>Botulism</td>
<td>Rapid descending pattern of progression; pupillary and autonomic involvement</td>
</tr>
<tr>
<td>Motor neuron disease</td>
<td>Presence of corticobulbar features, muscle cramps / fasciculations / atrophy, upper motor neuron signs</td>
</tr>
<tr>
<td>Mitochondrial disorders</td>
<td>Onset more gradual; no fluctuation; symmetric weakness; often no diplopia despite severe ophtalmoplegia</td>
</tr>
<tr>
<td>Acute inflammatory demyelinating</td>
<td>No fluctuation in weakness; areflexia; acute onset</td>
</tr>
<tr>
<td>polynueopathy variant syndromes</td>
<td></td>
</tr>
<tr>
<td>Thyroid ophthalmopathy</td>
<td>Proptosis</td>
</tr>
<tr>
<td>CNS disorders causing cranial nerve dysfunction</td>
<td>Sudden onset; consciousness, coordination, and sensation affected; ocular weakness in distribution of individual nerves</td>
</tr>
</tbody>
</table>
MG SUBGROUP CLASSIFICATION

Experts have recently proposed that MG patients should be classified according to antibody status, age at disease onset, presence of thymoma and course type into the following disease subgroups (12,24):

- **AchR-MG**
  - *Early onset* (disease onset before 50 years of age)
  - *Late onset* (disease onset after 50 years of age)
  - *Thymoma-associated MG*

- **MuSK-MG**

- **LRP4-MG**

- **Seronegative MG**

- **Ocular MG**

According to experts’ opinion these disease subgroups vary in terms of clinical characteristics, disease pathogenesis and response to therapies. Grouping patients in these MG subtypes would thus allow more tailored treatment (12, 24).

The characteristics of each MG subgroup relevant for clinical practice are summarized in the following section.

**AChR-MG.**

- *Early onset.* This subtype of MG includes patients with AChR antibodies, which develop generalized disease symptoms before the age of 50 years(12, 24). Thymic follicular hyperplasia occurs often and this subgroup of patients responds to thymectomy. Female cases outnumber male cases by the ratio of 3:1. Patients belonging to this subgroup have an increased frequency of organ specific and general autoimmune disorders, especially thyroiditis. 15-25% of patients with MG belong to this subgroup (12, 24).
o **Late onset.** This subgroup of MG includes patients with AChR antibodies, who develop generalized disease symptoms after the age of 50 years (12, 24). Thymic hyperplasia occurs only rarely in this subgroup and patients most often will not respond to thymectomy. The disease is slightly more frequently reported in males than females. 35-45% of patients with MG belong to this subgroup (12, 24).

o **Thymoma associated MG.** Thymoma associated MG is a paraneoplastic disease (12, 24). A thymoma is recorded in 10-15% of all patients with MG. Nearly all have detectable AChR antibodies and generalized disease. About 30% of patients with thymoma develop myasthenia gravis and another 15% have anti-AChR antibodies without MG symptoms. Tymectomy is absolutely indicated in this subgroup of patients (12, 24).

**MuSK-MG.** The presence of anti-MUSK antibodies is associated with more severe disease that predominantly involves oropharyngeal, facial, neck and respiratory muscles (12, 24). Limb weakness is less common than in AChR-MG, and ocular muscles may be spared. Little variation of muscle strength is reported during the day, and atrophy of affected muscles might occur. The risk of myasthenic crisis is particularly high, and the chances of achieving complete stable remission are significantly lower than in anti-AChR MG. The great majority of patients are middle-aged women. No thymus pathological changes are reported and patients usually have no response to thymectomy. Up to 10% of all MG patients belong to this subgroup (12, 24).

**LRP4-MG.** MG with LRP4 antibodies is less well characterized than the other disease subgroups (12, 24). Patients tend to have milder symptoms, both at initial presentation and over the course of the disease progression. Most of these patients present with ocular or generalized mild myasthenia, and about 20% of patients have only ocular weakness for more than 2 years. Respiratory insufficiency occurs very rarely. There is no association with thymic pathology and no benefit of thymectomy. Up to 5% of patients with MG belong to this subgroup. A few patients have been reported to have additional anti-AChR or anti-MUSK antibodies; these patients tend to
have more severe MG and should be categorized according to the relevant coexistent antibody subgroup. Commercial tests for LRP4 antibodies are not yet available, meaning that this subgroup can be identified only by a few institutions (12, 24).

**Seronegative MG.** The seronegative MG subgroup is heterogeneous, as it encompasses from patients with antibodies that have affinities or concentrations to low to detect to patients with antibodies against relevant antigens that have not yet been defined (12, 24).

The seronegative MG subgroup constitutes 10-15% of patients with generalized MG, depending on the sensitivity of the antibody test used. Repeated antibody testing can, in some patients, lead to a diagnostic revision from seronegative MG subgroup to one of the other subgroups owing to increased antibody concentration, epitope spreading, or increased test sensitivity. Repeated antibody testing in seronegative MG patients 6-18 months after the initial assessment is currently recommended (12, 24).

**Ocular MG** This subgroup encompasses patients with MG symptoms restricted to extraocular muscles, irrespective of the antibodies, thymic pathology (except thymoma) and disease duration (12, 24). The prevalence of ocular MG is similar among MG patients with anti-AChR and anti-LRP4 antibodies, whereas ocular MG with MuSK antibodies is nearly non-existent. Up to 15% of all MG patients belong to this subgroup (12, 24).
TREATMENT OF MG

The general management approach for MG includes early recognition, prompt treatment, support of bulbar/respiratory function, symptomatic treatment by pharmacologic enhancement of neuromuscular transmission, and immunotherapy. Treatment options include symptomatic, long or short acting immunosuppressive, and long or short acting immunomodulatory therapy (3, 33). The agents and procedures used for the treatment of MG are summarized in table 3, 4 and 5 (3, 12, 24, 33, 81, 90).

Treatment regimens should be individualized based on disease subtype and should take into account autoantibody status, thymic pathology, age, comorbidities, and disease severity. The goal of treatment is to restore the patient to fully functional status, and to maintain the improved condition without recurrence or adverse effects of the treatment (78).

Symptomatic treatments (Cholinesterase inhibitors)

Oral cholinesterase inhibitors (table 3) increase the amount of acetylcholine available for binding in the NMJ, and are the first-line treatment in all types of autoimmune MG (85, 91). Pyridostigmin bromide is the most commonly used cholinesterase inhibitor. The initial dose is 30-60mg every 4-6h, which is increased and adjusted to maximize benefit and minimize side-effects (diarrhea, stomach cramps). Neostigmin can be given 30-60min before meals in patients with bulbar symptoms (78). Cholinesterase inhibitors only rarely induce complete or sustained relief of MG symptoms. It is important to understand that treatment with cholinesterase inhibitors does not affect disease progression or outcome and will not prevent a patient with severe bulbar or respiratory muscle weakness from experiencing a worsening of symptoms leading to a severe exacerbation or even crisis (78).
However, treatment with cholinesterase inhibitors might be sufficient for adequate management in certain patients with non progressive mild or purely ocular disease. Doses of pyridostigmin exceeding 360mg daily are rarely effective and potentially dangerous since these higher doses can induce worsening muscle weakness due to depolarization block of neuromuscular transmission (78). Cholinergic overdose is often (but not always) accompanied by the muscarinic symptoms of hypersalivation, bradycardia, hyperhidrosis, lacrimation, and miosis (15).

It is worth to mention that patients with MuSK-MG may not respond to cholinesterase inhibitors and higher doses may actually lead to increased weakness. This phenomenon is thought to be due to desensitization of AChRs (92).

**Table 3: Symptomatic treatments of MG (3, 78, 81, 90, 91)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage/frequency</th>
<th>Onset of action</th>
<th>Serious adverse events</th>
<th>Monitor</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridostigmine bromide</td>
<td>30-60 mg p.o. every 4-6 hours</td>
<td>30 to 45 min</td>
<td>Cholinergic crisis</td>
<td>/</td>
<td>First line treatment; risk of cholinergic crisis increased in dosage exceeding 120mg/4 h (89)</td>
</tr>
<tr>
<td>(Mestinon®)</td>
<td>(maximal daily dose: 960 mg)</td>
<td>wearing off 3-6 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambenonium chloride</td>
<td>5-10 mg p.o. every 6-8 hours</td>
<td>30-45 min; wearing off 3-6 h</td>
<td>Cholinergic crisis</td>
<td>/</td>
<td>Use in patients untollerant or unresponsive to pyridostigmine (89)</td>
</tr>
<tr>
<td>(Mytelase®)</td>
<td>(maximal daily dose: 200 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neostigmin</td>
<td>1.5 mg i.m. every 4-6 hours</td>
<td>20-30 min; 4-8 min; wearing off 2-4h</td>
<td>Cholinergic crisis</td>
<td>/</td>
<td>First line treatment in patients unable to swallow</td>
</tr>
<tr>
<td>(Prostigmin®)</td>
<td>0.5 mg i.v. every 4-6 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(maximal daily dose: 10 mg)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Immunosuppressive treatments

Nearly all patients with late-onset MG, thymoma MG, and MuSK MG require immunosuppressive therapy to suppress autoantibody production and autoantibody-induced detrimental effects at the NMJ (12). Early-onset MG can sometimes be alleviated by symptomatic therapy alone, but the majority of patients with early-onset MG require pharmacological immunosuppression; however, in many of them, this need is temporary (12).

LRP4-MG is usually relatively mild, and immunosuppression is often not needed (12). For ocular MG, there are two treatment aims: to improve symptoms (ptosis and diplopia) and to prevent disease generalization. Immunosuppression can do both (12, 93).

First-line treatments. First line immunosuppressive drug therapy for MG comprises either methylprednisolone or the combination of methylprednisolone with azathioprine (12, 24, 33, 85, 91, 94). These drugs have a broad action on the immune system, and have been shown to be beneficial in all MG subgroups, though the benefit varies depending on the subgroup (12).

According to current recommendations, methylprednisolone alone should be given only as a short-term treatment (<1 year) (10, 12, 24, 33, 85, 91, 94). Long-term methylprednisolone monotherapy could be considered for the treatment of ocular MG, but for the majority of other patients with MG, combination immunosuppressive treatment is recommended to obtain maximum effect with minimal adverse effects (10, 12, 24, 33, 85, 91, 94).

A transient worsening of weakness may occur if methylprednisolone is started at a high dose (12, 85, 95). To avoid this, it is recommended that methylprednisolone should be started at a low dose on alternate days and gradually titrated upwards (12, 85, 95). Once the optimal improvement has been reached, the dose of methylprednisolone should be slowly reduced to the lowest effective dose (12, 85, 95).
Azathioprine (initiated at 50mg daily) can be used alone or as a steroid-sparing agent, but when used in combination with methylprednisolone it might be more effective and better tolerated than methylprednisolone alone (15, 94). In the absence of systemic side-effects, the dose is gradually titrated upward by 50mg per week to a daily dose of 2-3mg/kg. In 15-20% of patients, an idiosyncratic reaction with influenza-like symptoms, which requires the drug to be stopped, occurs within 10-14 days after starting azathioprine. Hepatotoxicity and leukopenia are also important adverse effects, but are reversible if detected early and the dose of azathioprine is reduced or discontinued (15). Patients with thiopurine methyl transferase deficiency cannot completely metabolise azathioprine, and a normal dose might lead to dangerous leukopenia (15). Measurement of thiopurine methyl transferase concentration is recommended before initiating azathioprine therapy, and is advisable with early or marked azathioprine associated leukopenia (15).

Controlled studies of immunosuppression in MG are scarce, and virtually none have described subgroup-specific effects (12). However, uncontrolled studies and clinical experience have proven the effect of this treatment for MuSK-MG, late-onset MG and thymoma MG, as well as for early-onset MG and LRP4-MG, which usually have milder symptoms (12). Whereas azathioprine takes 6-15 months to yield an optimal effect, methylprednisolone exerts its full effect during the first few weeks and months of treatment (12, 95).

Two studies have indicated that methylprednisolone treatment of ocular MG reduces the risk of MG generalization, in addition to the beneficial effect on the ocular symptoms (12, 96, 97). Any added value of azathioprine for ocular MG has not been shown (12).

If immunosuppressive therapy induces pharmacological remission or a marked improvement, it should be maintained for the long-term, but the dose should be reduced to avoid adverse effects (12, 24, 85, 91). Full drug withdrawal will often lead to new exacerbations, particularly in MuSK-MG, thymoma MG and late-onset MG (12, 24, 85, 91). The presence of additional antibodies, particularly against RyR and titin, is an indication for long-term treatment, as these antibodies are more common in severe MG (10, 12, 26). Methylprednisolone and azathioprine can be safely administrated during pregnancy and lactation (12, 98).
Second-line treatments. Priorities for second line immunosuppressive therapy are debated. No controlled studies have compared different drugs in MG. Formal studies, examining specific therapies in well defined MG cohorts, including different MG subgroups, are sparse (12). For moderate and mild MG, mycophenolate mofetil is an option after the failure of first-line therapy owing to insufficient benefit or problematic adverse effects (12, 85, 91, 99). Evidence that supports the use of this drug is strongest in AChR-MG (12, 99). Even though open studies and clinical experience point to an effect, two well-controlled studies failed to show an additional benefit of mycophenolate mofetil as an add-on to methylprednisolone (12, 100, 101). It should also be noted that mycophenolate mofetil carries a teratogenic risk and should be avoided in females of childbearing age (12, 98).

Rituximab has emerged as a potent drug in MG (10, 12, 24, 30, 31). This monoclonal antibody binds specifically to the B-lymphocyte surface antigen CD20. For severe MG and for MuSK-MG in particular, rituximab can be given if the first immunosuppressive therapy fails (102, 103). Uncontrolled and/or observational studies have shown that overall, more than 80% of patients with severe or refractory MG responded to rituximab (104). A controlled clinical trial of rituximab in anti-AChR MG is currently ongoing (NCT02110706) (33). However, optimal treatment regimens with rituximab for MG as a whole or for specific MG subtypes have not yet been defined. The major limiting factors in rituximab treatment of MG are the potential risk of JC-virus-related progressive multifocal leukoencephalopathy and the high cost of treatment (24).

Alternative second-line treatments and third-line treatments. Alternative second-line and third-line treatment options for MG include methotrexate, cyclosporine, tacrolimus and cyclophosphamide (10, 12, 85, 91). Due to potentially serious adverse events, the use of these drugs should be reserved for patients who are unresponsive or intolerant to first or above mentioned second-line line treatments.

Cyclosporine inhibits T-cell proliferation via disruption of calcineurin signaling, which blocks the synthesis of interleukin 2 and other proteins essential to the function of CD4 T cells (15). It’s efficacy in MG has been suggested by a small, randomized, placebo-controlled clinical trial (15, 104) and retrospective studies have supported its use as a steroid-sparing agent (15, 106). Side
effects are common and include hirsutism, tremor, gum hyperplasia, and, anemia, but hypertension and nephrotoxicity are the main treatment-limiting adverse reactions (15, 106). In addition, cyclosporine response rates for different MG subgroups have not been defined (12).

**Tacrolimus (FK506)** belongs to the same class of immunosuppressant medication as cyclosporine and has a similar mechanism of action (15, 24). It appears to be less nephrotoxic as compared to cyclosporine (15). The efficacy of tacrolimus in MG has been suggested by several reports of successful treatment (107-109), especially in anti-RyR-positive MG patients where sustained benefit of tacrolimus treatment has been reported (110). It has been hypothesized, that the beneficial effect of tacrolimus in this subgroup of MG patients is mainly due to the enhancement of ryanodine-receptor-related sarcoplasmic calcium release (110). A new trial comparing tacrolimus with placebo for patients with an insufficient response to corticosteroids (NCT01325571) has been completed in July 2016 with awaiting results.

**Methotrexate** is a structural analogue of folic acid and exerts an antiproliferative effect on immune cells by inhibiting DNA synthesis (10, 111). Both safety and efficacy of this drug is well documented in other autoimmune diseases such as rheumatoid arthritis, but poorly investigated in MG (10,111). A recently published randomized controlled trial of methotrexate vs placebo failed to show steroid-sparing benefit of the study drug in MG in months 4 to 12 of treatment (112). However, the trial had several methodological limitations, due to which experts suggest that this report should not be taken as a proof that methotrexate has no role in the treatment of at least some MG patients (113). As a consequence, methotrexate is still considered as a second line treatment option for MG patients who do not respond to first choice immunosuppressive drugs in the recently published International consensus guidance for management of myasthenia gravis and in the European Federation of Neurological Societies (EFNS) guidelines (10, 85, 91).

**Cyclophosphamide** is used in severe MG. It acts as an alkylating agent that interferes with DNA replication (10, 15). It is a strong suppressor of B-cell activity and antibody synthesis, and at high doses it also affects T-cells (10). The effect on MG is well-documented but with a risk of toxicity leading to bone marrow suppression, opportunistic infections, bladder toxicity, sterility and neoplasms. Due to this unfavorable adverse effects profile, its use should be reserved for patient unresponsive to other treatments (10, 15).
Table 4: Immunosuppressive treatments of MG (12, 15, 24, 33)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage/frequency</th>
<th>Onset of action</th>
<th>Serious adverse events</th>
<th>Monitor</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylprednisolone (PR)</td>
<td>4-64mg/every 1-2 days p.o.</td>
<td>3-6 weeks</td>
<td>Cushingoid features, diabetes, hypertension, osteoporosis, psychiatric disorders</td>
<td>BP, glucose, bone density</td>
<td>First line immunosuppression therapy; short-term use of high doses; frequent side effects</td>
</tr>
<tr>
<td>Azathioprine (AZA)</td>
<td>2-3mg/kg/day p.o.</td>
<td>6-12 months</td>
<td>Haematopoietic suppression, hepatotoxicity, malignancy, pancreatitis</td>
<td>CBC, liver function</td>
<td>First line steroid sparing drug</td>
</tr>
<tr>
<td>Mycophenolate mofetil (MM)</td>
<td>2.0-2.5g/day p.o. in divided twice-daily doses</td>
<td>4-12 months</td>
<td>Haematopoietic suppression, hepatotoxicity, opportunistic infections, PML</td>
<td>CBC</td>
<td>First line steroid sparing drug in mild to moderate disease</td>
</tr>
<tr>
<td>Ciclosporin (CyA)</td>
<td>4-6mg/kg/day p.o. in divided twice-daily doses</td>
<td>2-3 months</td>
<td>Hypertension, malignancy, nephrotoxicity</td>
<td>Renal function, BP</td>
<td>Steroid sparing in patient intolerant or unresponsive to AZA or MM</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>7.5-25 mg p.o. weekly</td>
<td>1-3 months</td>
<td>Haematopoietic suppression, hepatotoxicity, pneumonitis</td>
<td>CBC, renal/liver function, chest X-ray</td>
<td>Similar efficacy and tolerability to AZA</td>
</tr>
<tr>
<td>Tacrolimus (FK506) (TCR)</td>
<td>3-5mg/day p.o.</td>
<td>4-8 weeks</td>
<td>Hyperglycaemia, hypertension, malignancy, nephrotoxicity</td>
<td>Renal function, potassium, BP, tacrolimus levels</td>
<td>Steroid-sparing in patients intolerant or unresponsive to AZA, MM, or CyA</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>a) 500mg/m² i.v. monthly x 6 months</td>
<td>2-4 weeks</td>
<td>Bladder toxicity, haematopoietic suppression, infertility, malignancy, opportunistic infections</td>
<td>CBC, platelet, urine</td>
<td>Use in refractory/severe MG</td>
</tr>
<tr>
<td></td>
<td>b) 50mg/kg i.v. daily x 4 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituximab</td>
<td>a) 375mg/m² i.v. weekly x 4 weeks repeated as needed every 5 months</td>
<td>1-6 months</td>
<td>Neutropaenia, opportunistic infections, progressive multifocal leucoencephalopathy</td>
<td>CBC, liver function</td>
<td>Use in refractory/severe MG</td>
</tr>
<tr>
<td></td>
<td>b) two infusions 1g each i.v. 2 weeks apart every 6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BP = blood pressure, CBC = complete blood cell count, AZA = azathioprine, MM = mycophenolate mofetil, CyA = ciclosporin, MTX = methotrexate, TCR = tacrolimus
**Rapid short-term immunomodulatory treatments**

Intravenous immunoglobulins (IVIG) and plasma exchange (PE) are used for acute severe exacerbations in generalized MG, to optimize strength before surgery or for rapid short-term immunotherapy (33). PE effectively improves strength in most patients with severe MG (114, 115). IVIG are widely used for patients with exacerbating MG (33). Randomized controlled trials comparing IVIG with PE have shown no significant differences between the two in the treatment of an acute MG exacerbation (114), but PE may have a faster onset of action and is the treatment of choice in MG crisis (15, 33, 91).

All MG subgroups, even seronegative MG, respond in a similar way to IVIG and PE, implying that seronegative patients also have circulating antibodies that are pathogenic (12).
Table 5: Immunomodulation treatments used in MG (12, 15, 33, 91)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage/frequency</th>
<th>Onset of action</th>
<th>Serious adverse events</th>
<th>Monitor</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous immunoglobulins (IVIG)</td>
<td>2g/kg i.v. (over 2-5 days)</td>
<td>3-10 days</td>
<td>Aseptic meningitis, solute induced renal failure, thrombotic complications, volume overload</td>
<td>BP, renal/cardiac status</td>
<td>Use in exacerbating MG</td>
</tr>
<tr>
<td>Plasma exchange (PE)</td>
<td>4-6 exchanges on alternate days</td>
<td>1-5 days</td>
<td>Disturbance in acid-base homeostasis, hypocalcaemia, hypotension, infection, pneumothorax, thrombosis, volume overload</td>
<td>BP, volume status, coagulation parameters</td>
<td>Use in exacerbating MG; treatment of choice in myasthenic crisis</td>
</tr>
<tr>
<td>Thymectomy</td>
<td>/</td>
<td>Several months – up to 2 years</td>
<td>General risks of surgery</td>
<td>/</td>
<td>Indicated in patients with thymoma and early onset AChR-MG</td>
</tr>
</tbody>
</table>

BP = blood pressure

Long-term immunomodulatory treatment - Thymectomy

Based on the presumed role of the thymus gland in the development of MG, therapeutic removal of the thymus has been performed in MG for more than 70 years (15). Thymectomy can be performed either transsternally or by a video-assisted thoracoscopic (VATS) approach. One review from 2011 concluded VATS to be the preferred method because of better cosmetic result, reduced need for a postoperative medication and equivalent disease resolution (116). The effect of thymectomy is expected to occur within two years. Immunotherapy is often started before thymectomy and continued and tapered off as the effect of surgery appears (11).
Data from several class III observational studies (117-119) and a recent class I randomized controlled trial (120) have shown that thymectomy is beneficial in MG for non-thymomatous MG patients. However, not all MG patients have a beneficial response to thymectomy; the later appears to be related to disease subgroup. Thus, the current recommendations for thymectomy are based on subgroup of MG (10, 12, 85, 91, 120):

- **Early onset MG.** MGTX (NCT00294658), a recently completed multicenter, multiracial, single blinded clinical study, has provided the first class I evidence of the significant effect of thymectomy in early onset MG in terms of improvement of weakness, reduction of the daily dose of corticosteroid therapy, and improvement of the quality of life. The benefit appears to be greater if the procedure is performed early after symptoms onset. Thymectomy is therefore recommended in early onset MG, and an early intervention is preferred compared to later in the course of MG (10, 12, 85, 91, 120).

- **Late-onset MG.** Thymectomy is not recommended for late-onset MG with an atrophic thymus. However, a proportion of late-onset MG patients, usually those with disease onset at 60-65 years, have a hyperplastic thymus and a favorable response to thymectomy, similar to that seen in the early-onset disease subgroup. Overall, in late-onset MG, the status of the thymus gland and consequent response to thymectomy appears to be linked to the presence or absence of anti-titin and anti-RyR antibodies: patients with atrophic thymus and no response to thymectomy are usually anti-titin and anti-RyR antibody-positive, whereas patients with hyperplastic thymus and a favorable response to thymectomy usually lack anti-titin and anti-RyR antibodies (10, 12, 85, 91).

- **Thymoma-associated MG.** Thymectomy should be undertaken as an oncological intervention when a thymoma is detected or strongly suspected to avoid local compression and spread to the thoracic cavity. The response of MG to thymectomy
is variable, and improvement of MG symptoms is usually more limited than in early-onset MG (10, 12, 85, 91).

- **MuSK and LRP4-MG.** MuSK-MG and LRP4-MG have no proven connection to the thymus or thymus pathology. Case reports of single patients improving after thymectomy have been published, but owing to very scarce evidence, thymectomy is not recommended (10, 12, 85, 91).

- **Ocular MG.** Thymectomy has not been shown to prevent generalization in ocular MG, or to induce remission. Therefore, thymectomy is not recommended for ocular MG (10, 12, 85, 91).

- **Seronegative MG.** Thymectomy is not indicated in seronegative patients, except in cases where the subgroup diagnosis is revised after the detection of low affinity anti-AChR antibodies (10, 12, 85, 91).
SPECIAL THERAPEUTIC SITUATIONS

**Myasthenic crisis.** Myasthenic crisis is defined as weakness from MG that is severe enough to necessitate intubation for ventilator support or airway protection (120). It is estimated that one out of five patients with MG will suffer myasthenic crisis at some point during their illness (122, 123). Intubation is generally indicated if there is evidence of respiratory muscle fatigue with increasing tachypnea and declining tidal volumes, hypoxemia, hypercapnia, and difficulty handling secretions (15, 78).

A precipitating factor can be identified in most cases of myasthenic crisis and most commonly include one or more of the following: bronchopulmonary infections, aspiration, surgical procedures including thymectomy, corticosteroid-induced worsening, rapid tapering of immune modulators, and exposure to drugs that may increase myasthenic weakness (78) (table 6). Excessive dosing of cholinesterase inhibitors can potentially increase weakness due to depolarization blockade. In addition to weakness, signs of cholinergic hyperactivity will be present, such as excessive salivation, increased bronchial secretions, muscle fasciculations, and abdominal cramping. It is recommended practice to discontinue the use of cholinesterase inhibitors after intubation because they might complicate the management of airway secretions and are not needed to support vital functions (15, 78).

Because of its rapid onset of action, PE is the favored treatment for myasthenic crisis. Comparison studies suggesting that IVIG is similarly efficacious in myasthenic crisis generally used suboptimum PE regimens and did not compare the onset of response (15, 114). Other reports suggest that IVIG might be less effective than PE (124). Because the effect of PE is only temporary, longer-acting immunosuppressive treatments (usually high-dose corticosteroids) should be added to maintain a longer therapeutic effect (15).
The timing of extubation and factors predicting success are not well established, but one report indicates that atelectasis is the strongest predictor of the need for reintubation (125). Non-invasive mechanical ventilation using bilevel positive-pressure ventilation might reduce the need for intubation in myasthenic patients who have not developed hypercapnia (partial CO2 pressure>50 mmHg/ 6.66 kPa) (126, 127)
**Table 6: Medications that might exacerbate MG (15, 81, 91)**

<table>
<thead>
<tr>
<th>Drugs that adversely affect MG (absolutely contraindicated in MG)</th>
<th>Drugs that are likely to adversely affect MG (contraindicated in MG)</th>
<th>Drugs that may exacerbate weakness in MG (should be used with caution in MG)</th>
</tr>
</thead>
</table>
| * D-penicillamine  
  * Telithromycin | * Curare and related drugs  
  * Botulinum toxin  
  * Aminoglycosides (gentamycin, kanamycin, neomycin, streptomycin, tobramycin)  
  * Macrolides (erythromycin, azithromycin)  
  * Fluoroquinolones (ciprofloxacin, levofloxacin, norfloxacin)  
  * Quinine, quinidine, procainamide  
  * Interferon-alpha  
  * Magnesium salts (i.v. magnesium replacement) | * Calcium channel blockers  
  * Beta-blockers  
  * Lithium  
  * Iodinated contrast agents  
  * Statins (causal relationship in these cases might be questionable given the widespread use of these agents) |
**MG in pregnancy.** Pregnancy may affect the course of MG in an unpredictable way (128, 129). The severity of the weakness at the beginning of the pregnancy does not predict either remission or exacerbation (130, 131). Generally speaking, worsening of symptoms most frequently occurs in the first trimester, or in the first 3 to 4 weeks postpartum, mainly due to stress and new demands (24). Recently published International consensus guideline for management of MG recommend planning for pregnancy well in advance to allow time for optimization of myasthenic clinical status and to minimize risks for the fetus (91). Multidisciplinary communication among relevant specialists should occur throughout pregnancy, during delivery, and in the postpartum period. Provided that their MG is under good control before pregnancy, the majority of women can be reassured that they will remain stable throughout pregnancy. Spontaneous vaginal delivery should be the objective and is actively encouraged (91).

Oral pyridostigmine is the first-line treatment during pregnancy, whereas intravenous cholinesterase inhibitors may produce uterine contractions, due to which their use is not recommended during pregnancy (91).

If immunosuppression is needed, corticosteroids are considered the agents of choice, since they do not increase the risk for fetal malformations or delayed fetal development (91). Current information indicates that azathioprine and cyclosporine are also relatively safe and could be used in pregnant women who are not satisfactorily controlled with or cannot tolerate corticosteroids (91). The use of other immunosuppressive agents is not recommended due to sparse evidence of teratogenicity (91).
PE and IVIG are considered a safe treatment option during pregnancy, when a prompt, although temporary response is required (i.e. in MG exacerbations or as a preparation for delivery) (91).

In case of eclampsia, the use of magnesium sulfate is not recommended due to its neuromuscular blocking effects; barbiturates or phenytoin usually provide adequate treatment (91).

Most female patients with MG give birth in an uncomplicated way. Apart from the risk of neonatal MG, no precautions are usually needed.

Lactation should be encouraged in patient with MG, also for women on immunosuppressive drugs (24, 132), but the passage of some medication into breast milk should be taken into account (24).
Fetal and transient neonatal MG. Approximately 10-15% of infants born to mothers with MG develop transient neonatal MG. Symptoms of transient neonatal MG usually develop a few hours after birth and, as the name suggests, are self-limited, resolving within 1 month. Treatment is supportive, including ventilator support and nasogastric feedings, if needed. Pyridostigmine (0.5mg/kg to 1.0mg/kg i.v.) in divided doses administered 30 minutes prior to feeding may be useful (15, 78).

Mothers with MG should always give birth at hospitals experienced in respiratory support treatment of newborn babies, because transient neonatal MG could develop even if the mother’s MG is well controlled. The cause of this transient muscular weakness is the transfer of the mother’s AChR or MuSK antibodies of the IgG class across the placenta (15, 78).

Transplacental AChR antibodies can, in rare instances, produce arthrogryposis due to severe intrauterine movement inhibition. Arthrogryposis, AChR-antibody induced stillbirths, and repeated spontaneous abortions can be avoided by IVIG infusions or PE before and during pregnancy. This treatment should be given to all women with MG who have already experienced such a pregnancy outcome (15).
PROGNOSIS

According to data from epidemiological studies, 77-82% of patients reach maximum weakness in the first 2-3 years of MG. With the current treatment possibilities, up to 13% of patients achieve complete remission of MG symptoms, 57% improve with treatment and 20% remain treatment refractory. The reported mortality rate ranges from 4-9%, which is considered the same as in the general population (7, 14).
MG IN SLOVENIA

During the years 2013 and 2014 we performed a retrospective analysis of the evaluation and treatment of all the newly diagnosed patients with MG in Slovenia between the years 2003 and 2013. This section reports the results of our analysis.

Number and geographical distribution of MG patients

According to data obtained from the archive of the Institute of Clinical Chemistry and Biochemistry of the University Medical Centre Ljubljana (database of the reference center for AChR-antibody assay testing for all Slovenian hospitals), archive of the Institute of Clinical Neurophysiology of the University Medical Centre Ljubljana (database of the reference center for neurophysiology testing for all Slovenian hospitals) and archives of all Slovenian hospitals with neurologic departments and/or neurology outpatient clinics, during the period from January 1, 2003 to December 31, 2013, Slovenian neurologist established the diagnosis of MG in 372 patients (187 (50.3%) women and 185 (49.7%) men; female to male sex ratio: 1.01:1). Of all the identified cases,

* 178 (48%) patients (92 women and 86 men) were diagnosed at the University Medical Centre Ljubljana,
* 77 (20%) patients 837 women and 40 men) were diagnosed at the University Medical Centre Maribor
* 30 (8%) patients (11 women and 19 men) were diagnosed in the General Hospital of Celje
* 29 (7.7%) patients (14 women and 15 men) were diagnosed in the General Hospital of Isola
* 19 (5%) patients (10 women and 9 men) were diagnosed in the General Hospital of Šempeter
* 18 (4.8%) patients (13 women and 5 men) were diagnosed in the General Hospital of Novo mesto
* 10 (2.7%) patients (4 women and 6 men) were diagnosed in the General Hospital of Slovenj Gradec
* 6 (1.6%) patients (3 women and 3 men) were diagnosed in the General Hospital of Jesenice
* 5 (1.3%) patients (3 women and 2 men) were diagnosed in the General Hospital of Murska Sobota.
Patients with MG were present in all the geographical regions of Slovenia. Their distribution, based on the postal code of the city of residence is shown in figure 7.

**FIGURE 7**: Geographical distribution of patients diagnosed with MG in Slovenia between January 1, 2003 and December 31, 2013, based on the postal code of the city of residence. N= number of patients.

The number of newly diagnosed MG patients per year ranged from 16 (in the year 2003) to 52 (in the year 2011) with a trend of increment.

**Age and gender characteristics of MG patients**

The age at onset of MG ranged from 14 to 91 years (median 62; IQR 45-72 years). Women had a significantly younger median age at onset (median 56.5; IQR 38.25-72 years) than men (median 62; IQR 45-72 years); p=0.0008. In the group with disease onset under 20 years of age, there was a net female predominance (5:1), in the group with disease onset between between 20 and 50 years of age the female
predominance still persisted (1.5:1), whereas in the group with disease onset above 50 years of age men predominated with a sex ratio of 1.3:1.

The median of age at diagnosis increased from 62 (IQR 29.5-73.5) years in 2003 to 69 (IQR 52-77.5) years in 2013. This increment was higher for women, where the median of age at diagnosis increased from 52 (IQR 25-72) years in 2003 to 71 (IQR 54.25-78) years in 2013. In men, the raise in age at the time of diagnosis was less pronounced during the study period (the median of age at diagnosis increased from 65 (IQR 45-73.5) years in 2003 to 69 (IQR 49.5-77) years in 2013).

**Antibody status of MG patients**

In the study’s population, 221 (59.4%) patients were AChR antibody positive, 121 (32.5%) patients were AChR antibody negative and for 30 (8.1%) patients no data on antibody status were available. In the subgroup of patients without AChR antibodies, few patients were tested for MuSK antibodies of which 2 (0.5%) patients resulted positive.

**Comorbidities of MG patients**

Thymoma was present in 30 (8%) patients, 43(11.6%) patients had other malignant diseases, 69 (18.5%) patients had thyroid diseases, 24 (6.4%) had other autoimmune diseases and 233 (62.6%) patients had other chronic diseases.

The majority of the patients presented within one year of symptoms onset: 56 (15%) patients presented within 1 week, 104 (28%) patients presented within 1 month, and 123 (33%) patients presented between 1 and 12 months after symptoms onset; longer disease duration was present in 89 (24%) patients.
On first presentation, 206 (55.4%) patients had isolated ocular disease, 162 (43.5%) patients had mild to moderate generalized disease symptoms, and 4 (1%) patients had severe generalized MG.

During the observation period, the disease remained confined to extra-ocular muscles in 138 (37%) patients. Of the remaining patients, 115 (31%) developed mild generalized disease, 56 (15%) developed moderate generalized disease, 32 (9%) developed severe MG and 31 (8%) experienced myasthenic crisis. The median of time from first symptoms onset to the development of myasthenic crisis was 17.5 (IQR 11-36.75) months.

**Analysis of treatment**

In the whole patients' population, 312 (84%) subjects received some kind of treatment, whereas 60 (16%) subjects were not treated, mainly due to a combination of very mild disease symptoms and the appearance of unsustainable side effects on any treatment attempt.

The majority of the patients were treated with symptomatic therapy with or without the addition of immunosuppressive therapy (i.e. 163 (44%) patients received only symptomatic therapy (cholinesterase inhibitors) and 94 (25%) patients were treated with a combination of symptomatic and immunosuppressive therapy), while 55 (15%) patients were treated predominantly with immunosuppression). The agents used for immunosuppression with the respective numbers and percentages of treated patients are summarized in table 7.
**Table 7:** Immunosuppressive treatment of MG patients in Slovenia between the years 2003-2013.

<table>
<thead>
<tr>
<th>Immunosuppressive therapy</th>
<th>Number of treated patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>91 (61%)</td>
</tr>
<tr>
<td>Azathiporine</td>
<td>15 (10%)</td>
</tr>
<tr>
<td>Corticosteroids + Azathiporine</td>
<td>9 (6%)</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>13 (8%)</td>
</tr>
<tr>
<td>Corticosteroids + Mycophenolate mofetil</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Corticosteroids + Rituximab</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td>Corticosteroids + Tacrolimus</td>
<td>7 (5.7%)</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>2 (1.3%)</td>
</tr>
<tr>
<td>Cyclosporine + corticosteroids</td>
<td>2 (1.3%)</td>
</tr>
</tbody>
</table>

**Outcome**

Upon treatment, 48 (15%) patients reached complete stable remission, 41 (13%) patients obtained pharmacologic remission, 186 (60%) patients substantially improved on treatment, 11 (4%) patients remained symptomatic despite treatment and 26 (8%) patients died. In the fatal cases, death was a direct consequence of MG in 11 (43%) cases (namely 7 (29%) patients died due to myasthenic crisis and 4 (14%) patients died due to adverse events of treatment), whereas in 15 (57%) cases, death was consequence of causes unrelated to MG (i.e. mainly complications of other chronic diseases).
AIM OF THE STUDY

The clinical course of MG is variable, ranging from remission in an early stage to severe weakness and even death (9, 133). Treatment strategy may differ for patients with severe disease, and it is therefore important to identify them as early as possible. At present there is no known prognostic factor that would predict the long-term clinical course in MG patients at the time of diagnosis.

SFEMG is considered the most sensitive diagnostic tool for diagnosis of MG (134, 135), but opinions differ regarding the prognostic value of the initial SFEMG data for the long-term clinical course. Some clinicians believe that MG patients with highly abnormal SFEMG results have a higher risk of developing severe disease, but many others doubt that this is a prognostic factor. However, objective evidence to support or refute this hypothesis is still lacking.

The aim of this study was to determine if the initial SFEMG values in the orbicularis oculi muscle (OOc) in MG are predictive of the severity of the long-term clinical course of the disease.

STUDY HYPOTHESIS

The extent of the initial SFEMG abnormalities of the OOc (namely mean jitter value, percentages of motor end-plates with increased jitter and/or impulse blocking) correlates with the severity of the later clinical course of MG.

Patients with higher mean jitter value, higher percentages of motor end-plates with increased jitter and/or impulse blocking on initial SFEMG of the OOc have a greater probability to develop severe exacerbations of myasthenia gravis.
MATERIALS AND METHODS

Study protocol

In this retrospective observational cohort study, we reviewed medical files of MG patients, diagnosed at the University Medical Centers of Ljubljana and Maribor, between January 1, 2003 and December 31, 2012.

We collected data on patients’ demographic characteristics, initial SFEMG parameters (mean jitter value, percentage of fibers with increased jitter and impulse blocking), AchR antibody status, presence of thymoma, thyroid pathology, other autoimmune, malignant or chronic disease, thymectomy status and use of immunosuppression. We correlated the patients’ initial SFEMG parameters and other clinical characteristics with their later disease course. The clinical stage was graded at the time of the patients' first presentation and at the time of maximal worsening according to the Myasthenia Gravis Foundation of America (MGFA) Clinical classification score (135) as follows:

- **score I**- disease restricted to ocular muscles
- **score II**- mild weakness affecting limb, axial or bulbar muscles, not interfering with activities of daily living and responsive to cholinesterase inhibitors and/or low dose immunosuppressive drugs
- **score III**- moderate weakness, which partially affects activities of daily living, responsive to immunosuppression with or without cholinesterase inhibitors
- **score IV**- severe weakness affecting limb, axial or bulbar muscles which incapacitates activities of daily living and requires treatment with plasma exchange (P.E.) or intravenous immunoglobulins (I.V.I.G.)
- **score V**- need for intubation, with or without mechanical ventilation.
The disease severity was then defined by the worst MGFA clinical classification score reached by the patient during the observation period.

According to the worst observed MGFA clinical classification score, the patients were divided into 2 outcome groups: Group 0, considered benign (patients who reached the worst MGFA clinical classification score of I-III), and Group 1, considered severe (patients who reached the worst MGFA clinical classification score of IV-V). The two outcome groups of patients were then compared for SFEMG parameters and other variables that could potentially influence the outcome (sex, age, time to symptoms onset to SFEMG, AchR antibody status, presence of thymoma, thyroid pathology, other autoimmune, malignant and/or chronic diseases).

Patients

Eligible patients fulfilled all of the following criteria:

- had the diagnosis of MG based on the clinical picture of fluctuating muscle weakness and fatigability in association with increased jitter and/or impulse blocking on SFEMG and positive response to acetylcholinesterase inhibitors and/or positive acetylcholine receptor (AchR) antibody assay
- had SFEMG of the OOc at the time of diagnosis
- had a MGFA Clinical classification score I or II at the time of first presentation/diagnosis
- had been followed up for at least 12 months.

The key exclusion criteria were:

- disease clinical stage rated III or more according to the MGFA Clinical classification score at the time of first presentation/diagnosis
- use of symptomatic or immunosuppressive therapy prior to diagnosis.
SFEMG

Jitter and impulse blocking were measured in the OOc using the stimulated SFEMG technique (137). Stimulation was performed with a near-nerve needle cathode in the facial branch to the muscle. Recording was performed with a single fiber needle electrode (SFE), inserted into the orbital portion of the orbicularis oculi muscle, about 10 mm lateral to the outer canthus, just at the margin of the orbit, at a distance of about 15-25 mm above and medially from the tip of the stimulating cathode (figure 8) (138).

Jitter was expressed as the mean consecutive difference (MCD) of 30 fibers. A study was considered abnormal if either of the following criteria were met: (1) the mean MCD (jitter) exceeded the upper limit of normal (ULN) for OOc (MCD > 20µs); or (2) more than 10% of individual fibers had an MCD greater than the ULN (30µs for individual end-plates) (137, 139).
FIGURE 8. The approximate position of the stimulating and recording needle electrodes used in the stimulated SFEMG technique (138)
**AChR autoantibody assay**

Serum IgG anti-AChR antibodies were tested in all patients on samples collected at the time of diagnosis. Autoantibodies were measured by radioimmunoprecipitation according to manufacturer’s instructions (IBL GmbH, Hamburg, Germany). A value of >0.4nmol/L was considered positive.

**Statistical analyses**

Unless otherwise indicated, demographic characteristics were indicated by the mean ± standard deviation or median and interquartile range (IQR) with respective 95% confidence intervals for continuous variables (for example, age). Proportions and 95% exact binomial based confidence intervals for proportions were estimated for categorical variables (for example, gender).

The difference between two groups for continuous variables was tested with $t$-test, Welsch $t$-test, or Mann-Whitney test, where appropriate. The assumption of normality was verified with the Shapiro-Wilk test, and the Bartlett test was used to test the assumption of variance equality.

The association between 2 categorical variables (for example, sex and outcome group) was tested with the $\chi^2$ test with Yates continuity correction of the Fischer exact test, where appropriate.

The association between SFEMG parameters was estimated with Pearson correlation coefficients.

Logistic regression was used to estimate the association between the SFEMG parameters and the outcome group. The Hosmer and Lemeshow test was used to test the goodness of fit of the model.
The cut-off values of SFEMG parameters were estimated by calculating the g-means (defined as geometric mean of sensitivity and specificity). Sensitivity and specificity were calculated with leave-one-out cross-validation from separate univariate logistic regression models.

A $P$-value of $<0.05$ was considered to be statistically significant. The analysis was performed with R language for statistical computing (R version 3.0.1) (140).

**Standard protocol approvals**

The study protocol was approved by the National Medical Ethics Committee of Slovenia (date of certificate release 19.3.2013; certificate nr.204/03/13 (Appendix 1)). Informed consent was waived.
RESULTS

The study group consisted of 232 patients with MG. At the time of diagnosis, when SFEMG was performed, all patients were in Class I or II of the MGFA Clinical Classification Score. During the observation period, 193 patients (83%) had a worst MGFA Clinical Classification Score of I-III and were thus classified into group 0, whereas 39 patients (17%) reached the worst MGFA Clinical Classification Score of IV-V and were therefore classified into group 1. The main clinical features of both groups of patients are outlined in Table 8.
Table 8: Clinical features of 232 patients with myasthenia gravis (MG) included in the study.

<table>
<thead>
<tr>
<th></th>
<th>Group 0 (worst MGFA Clinical Classification Score I-III)</th>
<th>Group 1 (worst MGFA Clinical Classification Score IV-V)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects (%)</td>
<td>193 (83)</td>
<td>39 (17)</td>
<td></td>
</tr>
<tr>
<td>No. of subjects with ocular symptoms only at first presentation</td>
<td>122 (63)</td>
<td>8 (21)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>104 (54)</td>
<td>21 (54)</td>
<td>0.8638</td>
</tr>
<tr>
<td>Sex ratio, F:M</td>
<td>1.17:1</td>
<td>1.17:1</td>
<td></td>
</tr>
<tr>
<td>Age at disease onset</td>
<td>55.72±17.81</td>
<td>64.44±16.98</td>
<td>0.0032</td>
</tr>
<tr>
<td>(mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from symptoms</td>
<td>5; 1-12</td>
<td>2; 1-6</td>
<td>0.0150</td>
</tr>
<tr>
<td>onset to SFEMG;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Median; Q1-Q3 (months))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AchR antibody status</td>
<td></td>
<td></td>
<td>0.0046</td>
</tr>
<tr>
<td>Positive (%)</td>
<td>111 (59.04)</td>
<td>33 (84.62)</td>
<td></td>
</tr>
<tr>
<td>Thymoma</td>
<td>10 (5.24)</td>
<td>3 (7.89)</td>
<td>0.4571</td>
</tr>
<tr>
<td>Thyroid pathology</td>
<td>32 (16.58)</td>
<td>8 (20.51)</td>
<td>0.7184</td>
</tr>
<tr>
<td>Other autoimmune</td>
<td>18 (9.33)</td>
<td>4 (10.26)</td>
<td>0.7708</td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other malignant disease</td>
<td>16 (8.29)</td>
<td>4 (10.26)</td>
<td>0.7536</td>
</tr>
<tr>
<td>Other chronic disease</td>
<td>111 (57.51)</td>
<td>20 (51.28)</td>
<td>0.5900</td>
</tr>
<tr>
<td>Thymectomy</td>
<td>31 (16.32)</td>
<td>6 (15.38)</td>
<td>0.9244</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>70 (36.27)</td>
<td>35 (89.74)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of the follow up time</td>
<td>33.73; 13.7-58.27</td>
<td>34.33; 23.57-58.98</td>
<td>0.2402</td>
</tr>
<tr>
<td></td>
<td>(median; Q1-Q3 (months))</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group 0=patients with benign clinical course (worst MGFA clinical classification score of I-III); Group 1= patients with severe clinical course (worst MGFA clinical classification score of IV-V); MGFA= Myasthenia Gravis Foundation of America; SD= standard deviation; Q=quartile; AChR=acetylcholine receptor
Univariate analysis showed no statistically significant differences in sex frequencies (p=0.8638), thymoma presence (p=0.4571), thymectomy status (p=0.9244), and median duration of follow-up time (p=0.2402) between the two outcome groups of patients.

However, patients from group 1 (severe clinical course) were statistically significantly older (p=0.0032), had a shorter time from symptom onset to SFEMG (p=0.0150), were less likely to have isolated ocular disease symptoms (p=0.0000), were more likely to be AchR-antibody positive (p=0.0046), and were more likely be treated with immunosuppression (p<0.0001).

Comparison for the presence of other comorbidities did not show any significant difference between the two outcome groups of patients in terms of thyroid disease (p=0.7184) or other autoimmune (p=0.7708), malignant (p=0.7536), or chronic diseases (p=0.59); (table 8).

There was a net difference between the two outcome groups of patients when they were compared for SFEMG parameters. The main SFEMG characteristics for each outcome group are summarized in Table 9.
### Table 9: SFEMG characteristics of 232 patients with MG

<table>
<thead>
<tr>
<th></th>
<th>Group 0 (worst MGFA Clinical Classification Score I-III)</th>
<th>Group 1 (worst MGFA Clinical Classification Score IV-V)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n (%)</td>
<td>193 (83)</td>
<td>39 (17)</td>
<td></td>
</tr>
<tr>
<td>Median of mean jitter</td>
<td>37; 26-66</td>
<td>95.5; 73-124.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IQR (μs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median percentage of</td>
<td>43; 18-83</td>
<td>94; 83-100</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>end-plates with increased jitter, IQR (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median percentage of</td>
<td>10; 5-30</td>
<td>47.5; 23.5-60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>end-plates with impulse blocking, IQR (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Group 0=patients with benign clinical course (worst MGFA clinical classification score of I-III); Group 1= patients with severe clinical course (worst MGFA clinical classification score of IV-V); MGFA= Myasthenia Gravis Foundation of America; IQR=interquartile range*

Univariate analysis showed that patients from group 1 had a statistically significant higher mean jitter value (p<0.0001) and a greater degree of motor end-plates with increased jitter (p<0.0001) and/or impulse blocking (p<0.0001).
The association between initial SFEMG characteristics and later clinical outcome was confirmed by logistic regression analysis. In the univariate models, higher mean jitter (OR=1.0253; 95% CI: 1.0373-1.0347; table 10), higher percentage of increased jitter (OR=1.0426; CI: 1.0258-1.0597; table 11), and impulse blocking (OR=1.0373, 95% CI:1.0226-1.0521; table 12) on the individual motor end-plates correlated with a worse later MGFA clinical classification score ($P<0.0001$).

**Table 10: Results of the logistic regression model for the variable “mean jitter value”**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th>z value</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-3.39236</td>
<td>0.42024</td>
<td>-8.07246</td>
<td>0.00000</td>
<td>0.03363</td>
<td>0.01476-0.07664</td>
</tr>
<tr>
<td>Mean jitter value</td>
<td>0.02499</td>
<td>0.00464</td>
<td>5.38967</td>
<td>0.00000</td>
<td>1.02530</td>
<td>1.01603-1.03466</td>
</tr>
</tbody>
</table>

Intercept = a mathematical constant with no clinical interpretation; Estimate = the mathematical weighting of each variable in the model; S.E.= standard error: the estimated error of the mathematical weighting; z value= Estimate/S.E.: a z value >2 indicates a significant association of the variable with the outcome; CI = confidence interval.

**Table 11: Results of the logistic regression model for the variable “percentages of motor end-plates with increased jitter”**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th>z value</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-4.54530</td>
<td>0.70473</td>
<td>-6.44968</td>
<td>0.00000</td>
<td>0.01062</td>
<td>0.00267-0.04226</td>
</tr>
<tr>
<td>% of motor end-plates with increased jitter</td>
<td>0.04173</td>
<td>0.00828</td>
<td>5.03735</td>
<td>0.00000</td>
<td>1.04261</td>
<td>1.02582-1.05968</td>
</tr>
</tbody>
</table>

Intercept = a mathematical constant with no clinical interpretation; Estimate = the mathematical weighting of each variable in the model; S.E.= standard error: the estimated error of the mathematical weighting; z value= Estimate/S.E.: a z value >2 indicates a significant association of the variable with the outcome; CI = confidence interval; % = percentages.
Table 12: Results of the logistic regression model for the variable “percentages of motor end-plates with conduction blocks”

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th>z value</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-2.75366</td>
<td>0.32676</td>
<td>-8.42713</td>
<td>0.00000</td>
<td>0.06369</td>
<td>0.03357-0.12085</td>
</tr>
<tr>
<td>% of motor end-plates with conduction blocks</td>
<td>0.03658</td>
<td>0.00725</td>
<td>5.04722</td>
<td>0.00000</td>
<td>1.03725</td>
<td>1.02262-1.05209</td>
</tr>
</tbody>
</table>

Intercept = a mathematical constant with no clinical interpretation; Estimate = the mathematical weighting of each variable in the model; S.E. = standard error: the estimated error of the mathematical weighting; z value = Estimate/S.E.: a z value >2 indicates a significant association of the variable with the outcome; CI = confidence interval; % = percentages.

For the multivariate logistic regression analysis we made three multivariate logistic regression models considering separately each SFEMG parameter with other variables that were statistically significant in the univariate analysis. The reason for such an approach was a strong association between SFEMG parameters shown by the Pearson correlation coefficients (r=0.799-0.872; P<0.0001; figure 9), due to which a single multivariate model could lead to invalid conclusions.
FIGURE 9: Correlation between SFEMG parameters in the whole population of patients.

Correlation between mean jitter value and proportion of motor end-plates with increased jitter (A), correlation between mean jitter value and proportion of motor end-plates with conduction blocks (B), correlation between proportion of motor end-plates with conduction blocks and proportion of motor end-plates with increased jitter (C). $r =$ Pearson's correlation coefficients; CI = confidence intervals; % = percentages.
Multivariate logistic regression analysis showed that SFEMG parameters also remained significantly associated with the outcome when adjusting for the effect of other variables that were statistically significant in the univariate analysis. Higher mean jitter (\(P=0.0025; \text{OR}=1.01630, 95\% \text{CI}:1.00566 -1.02704; \text{table 13}\)), higher percentage of fibers with increased jitter (\(P=0.0010; \text{OR}=1.01271-1.05146; \text{table 14}\)), and/or impulse blocking (\(P=0.0053, \text{OR}=1.02377, \text{CI}:1.00697-1.04085; \text{table 15}\)) in individual motor end-plates were predictive of a severe clinical course.

**Table 13: Results of multivariate logistic regression model considering »mean jitter value« as an independent SFEMG parameter: Predictors of disease clinical course**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th>z value</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-5.68607</td>
<td>1.04286</td>
<td>-5.45239</td>
<td>0.00000</td>
<td>0.00339</td>
<td>0.00044-0.02620</td>
</tr>
<tr>
<td>AchR antibody status</td>
<td>0.42722</td>
<td>0.54119</td>
<td>0.78942</td>
<td>0.42987</td>
<td>1.53299</td>
<td>0.53073-4.42800</td>
</tr>
<tr>
<td>Age</td>
<td>0.02975</td>
<td>0.01219</td>
<td>2.44016</td>
<td>0.01468</td>
<td>1.03020</td>
<td>1.00587-1.05511</td>
</tr>
<tr>
<td>Mean jitter value</td>
<td>0.01616</td>
<td>0.00536</td>
<td>3.01293</td>
<td>0.00259</td>
<td>1.01630</td>
<td>1.00566-1.02704</td>
</tr>
<tr>
<td>Time from symptoms onset to SFEMG</td>
<td>-0.00099</td>
<td>0.01659</td>
<td>-0.05944</td>
<td>0.95260</td>
<td>0.9901</td>
<td>0.96706-1.03202</td>
</tr>
<tr>
<td>Presence of extra ocular disease symptoms at the time of first presentation</td>
<td>1.43911</td>
<td>0.48147</td>
<td>2.98900</td>
<td>0.00280</td>
<td>4.21695</td>
<td>1.64121-10.83510</td>
</tr>
</tbody>
</table>

*Intercept = a mathematical constant with no clinical interpretation; Estimate = the mathematical weighting of each variable in the model; S.E. = standard error: the estimated error of the mathematical weighting; z value = Estimate/S.E.: a z value >2 indicates a significant association of the variable with the outcome; CI = confidence interval; AchR = acetylcholine receptor; SFEMG = single fiber electromyography.*
**Table 14:** Results of multivariate logistic regression model considering “percentages of motor end-plates with increased jitter” as an independent SFEMG parameter: Predictors of disease clinical course

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th>z value</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>-6.17087</td>
<td>1.10423</td>
<td>-5.58839</td>
<td>0.00000</td>
<td>0.00209</td>
<td>0.00024-0.01820</td>
</tr>
<tr>
<td>AchR antibody status</td>
<td>-0.15393</td>
<td>0.60394</td>
<td>-0.25487</td>
<td>0.79882</td>
<td>0.85733</td>
<td>0.26246-2.80051</td>
</tr>
<tr>
<td>Age</td>
<td>0.02863</td>
<td>0.01201</td>
<td>2.38490</td>
<td>0.01708</td>
<td>1.02905</td>
<td>1.00511-1.05355</td>
</tr>
<tr>
<td>% of motor end-plates with increased jitter</td>
<td>0.03141</td>
<td>0.00958</td>
<td>3.27902</td>
<td>0.00104</td>
<td>1.03190</td>
<td>1.01271-1.05146</td>
</tr>
<tr>
<td>Time from symptoms onset to SFEMG</td>
<td>-0.00232</td>
<td>0.01598</td>
<td>-0.14529</td>
<td>0.88448</td>
<td>0.99768</td>
<td>0.96691-1.02943</td>
</tr>
<tr>
<td>Presence of extra ocular disease symptoms at the time of first presentation</td>
<td>1.37446</td>
<td>0.48626</td>
<td>2.82660</td>
<td>0.00470</td>
<td>3.95292</td>
<td>1.52408-10.25250</td>
</tr>
</tbody>
</table>

*Intercept = a mathematical constant with no clinical interpretation; Estimate = the mathematical weighting of each variable in the model; S.E. = standard error: the estimated error of the mathematical weighting; z value = Estimate/S.E.: a z value >2 indicates a significant association of the variable with the outcome; CI = confidence interval; AchR = acetylcholine receptor; % = percentages; SFEMG = single fiber electromyography.*
Table 15: Results of multivariate logistic regression model considering »percentages of motor end-plates with conduction blocks« as an independent SFEMG parameter: Predictors of disease clinical course

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th>z value</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>-5.51563</td>
<td>1.03217</td>
<td>-5.34372</td>
<td>0.00000</td>
<td>0.00402</td>
<td>0.00053-0.03042</td>
</tr>
<tr>
<td>AchR antibody status</td>
<td>0.53122</td>
<td>0.53645</td>
<td>0.99025</td>
<td>0.32205</td>
<td>1.70100</td>
<td>0.59439-4.86788</td>
</tr>
<tr>
<td>Age</td>
<td>0.03091</td>
<td>0.01212</td>
<td>2.55047</td>
<td>0.01076</td>
<td>1.03139</td>
<td>1.00718-1.05618</td>
</tr>
<tr>
<td>% of motor end-plates with conduction blocks</td>
<td>0.02349</td>
<td>0.00844</td>
<td>2.78311</td>
<td>0.00538</td>
<td>1.02377</td>
<td>1.00697-1.04085</td>
</tr>
<tr>
<td>Time from symptoms onset to SFEMG</td>
<td>-0.00042</td>
<td>0.01612</td>
<td>-0.02582</td>
<td>0.97940</td>
<td>0.99958</td>
<td>0.96850-1.03167</td>
</tr>
<tr>
<td>Presence of extra ocular disease symptoms at the time of first presentation</td>
<td>1.61507</td>
<td>0.46649</td>
<td>3.46217</td>
<td>0.00054</td>
<td>5.02824</td>
<td>2.01526-12.54589</td>
</tr>
</tbody>
</table>

Intercept = a mathematical constant with no clinical interpretation; Estimate = the mathematical weighting of each variable in the model; S.E. = standard error: the estimated error of the mathematical weighting; z value = Estimate/S.E.: a z value >2 indicates a significant association of the variable with the outcome; CI= confidence interval; AchR = acetylcholine receptor; % = percentages; SFEMG = single fiber electromyography.

Of the remaining variables that were significantly associated with the outcome in the univariate analysis, only higher patient age (P=0.015-0.018, OR=1.02905-1.03139, CI:1.00718-1.05511) and the presence of extraocular muscle weakness (P=0.0005-0.0047; OR=3.95292-5.02824, CI: 1.52408-12.54589) were predictive of a severe clinical course, whereas AchR-antibody status (P=0.29395-0.65824) and duration of symptoms prior to diagnosis (P=0.64622-0.82657) were not significantly associated with the outcome in the multivariate models (tables 13-15). The more extensive use of immunosuppression therapy in the patients with severe disease
exacerbations was considered a consequence of debilitating disease (note that all patients were treatment naïve at the time the SFEMG was performed). For that reason, the use of immunosuppression was not included in the multivariate logistic regression analysis.

After determining a significant association of the SFEMG parameters with the outcome, we estimated also their cut-off values that predicted a severe clinical course. These values were $\geq 68.61\mu s$ for mean jitter, $\geq 81.31\%$ of individual motor end-plates with increased jitter, and $\geq 21.86\%$ of individual motor end-plates with impulse blocking, respectively (Figure 10).

The respective cross-validated g-means (sensitivity, specificity) obtained with these cut-off values were 78% (77%, 79%), 77% (75%, 79%), and 74% (69%, 79%) for mean jitter, percentage of motor end-plates with increased jitter, and percentage of motor end-plates with impulse blocking, respectively. These values show good predictive ability of the SFEMG parameters.
FIGURE 10: Distribution of single fiber electromyography (SFEMG) parameters values between the two outcome groups of patients.

Mean jitter value (A), percentages of motor end-plates with increased jitter (B), percentages of motor end-plates with impulse blocking (C).

The horizontal line represents the optimal cut-off value as the cut-off that achieves the largest g-means for each parameter.

Group 0 = patients with benign clinical course (worst MGFA clinical classification score of I-III); Group 1 = patients with malignant clinical course (worst MGFA clinical classification score of IV-V).

MGFA = Myasthenia Gravis Foundation of America. Total number of patients: 232; number of patients in group 0: 193; number of patients in Group 1: 39.
DISCUSSION

In our retrospective observational cohort study on 232 patients with MG we found, that initial SFEMG of the OOc has prognostic value for the long-term clinical course of the disease. In the study population, severe disease exacerbations were observed in subjects with a mean jitter value greater than 68.6μs, at least 81.3% of motor end-plates with increased jitter, and/or at least 21.8% of motor end-plates with conduction block. On the other hand, patients with lower mean jitter value, lower percentage of motor end-plates with increased jitter, and/or conduction block had a more benign clinical course. This association persisted also when we adjusted for the effect of other clinical characteristics that could potentially influence the outcome according to multivariate logistic regression analysis (namely age and the presence of generalized disease symptoms).

Previous serial studies of MG have shown that in most patients, the change in SFEMG measurements correlated with the change in clinical state as measured by quantitative testing of muscle function (139, 141, 142). In these studies, MCD increased by at least 10% in the tested muscle in two-thirds of the patients who become clinically worse between consecutive SFEMG examinations. Conversely, in over 80% of instances in which mean MCD fell by at least 10% between two SFEMG evaluations, there was definite clinical improvement (137, 139, 141, 142). In a placebo-controlled, therapeutic, pilot trial of mycophenolate mofetil in MG, Meriglioli et al. confirmed the same decrease in MCD in patients with a positive response to
immunosuppression therapy, thus indicating a potential value of SFEMG as a marker of early treatment response (143).

To our knowledge, only 4 studies have investigated the prognostic value of SFEMG in MG patients (144-147). All of them addressed the ability of SFEMG of the extensor digitorum muscle (ED) to predict disease generalization in patients with ocular MG. Their results were conflicting.

The first, a retrospective study on 24 patients, showed that disease generalization was more likely to occur in patients with mean jitter values exceeding 50μs, whereas in patients with a lower mean jitter, the disease tended to remain confined to extra-ocular muscles (144). In the second, prospective study on 37 patients, a normal mean jitter was associated with MG remaining restricted to the extra-ocular muscles, but a higher mean jitter was not predictive of subsequent disease generalization (145). In the third and fourth retrospective studies made on 50 and 102 patients, respectively, SFEMG did not show any predictive value for later disease generalization in patients with isolated ocular disease (146, 147).

However, it should be emphasized, that all the above mentioned four studies were conceptually different from our study. The main differences were in the primary end point and in the muscle used for SFEMG evaluation. In contrast with previous studies, we used SFEMG to identify patients at risk for severe disease exacerbations. The choice of this primary end point was based on data from historical observational studies, which showed that up to 20% of patients with MG experience severe disease exacerbation during their lifetime (148). Identification of this subgroup at the time of diagnosis would allow for more intensive follow up and early initiation of effective immunosuppressive treatment. Accordingly, severe, potentially life-threatening disease exacerbation could possibly be prevented.
The choice of the muscle for SFEMG was a direct consequence of the differences in the primary end-point and tested hypothesis. In all previous studies, the main idea was that there is a qualitative difference in electrodiagnostic muscle involvement between patients with ocular MG destined to remain ocular and patients who later generalized (144-147). In contrast with patients destined to stay ocular, patients who later developed generalized disease were suspected to have subclinical electrodiagnostic involvement of other skeletal muscles. For this reason SFEMG was performed on the ED, which was felt to have increased mean jitter values only in patients who would later experience disease generalization. However, this supposition failed to be confirmed.

In our study, we tested a different hypothesis. Studies of normal and abnormal neuromuscular transmission with SFEMG showed that the extraocular muscles, and in particular the OOc, are electrodiagnostically affected in virtually all MG patients (139). However, despite being affected, the extraocular muscles are not always symptomatically weak (15). The susceptibility of extraocular muscles for MG pathology is probably the result of differences in neuromuscular junction morphology and physiology (they have less prominent synaptic folds, fewer postsynaptic AchRs, and smaller motor units that are subjected to high firing frequencies (15, 149, 150); in addition, they have a low expression of complement regulators that might make them more vulnerable to complement-mediated damage (15, 151)). In this context, the extent of changes in jitter could function as a surrogate marker of the autoimmune process at the level of the neuromuscular junction. Therefore, our main assumption was that a higher mean jitter value in the extra ocular muscles could be
potentially predictive of later severe disease exacerbations. Hence, we chose the OOc for SFEMG evaluation.

Another aspect of our data that deserves a special comment is the age of the patient population at disease onset. Since the mean age at disease onset was relatively high in both patient groups (55.7 years in group 0 and 64.4 years in group 1), it would seem that our patient population is too old to function as a model for long-term disease outcome. Nonetheless, in most MG patients the disease course is determined early (77-82% of patients reach maximum weakness in the first 2-3 years (7, 152)). In addition, several previous and recently published review articles and epidemiological studies have reported a continuously increasing incidence of MG in patients above age 50 years (24, 152-155). Therefore, the relatively high mean age at disease onset of our patient population should be considered both a representative sample and a consequence of the current epidemiology of MG. Moreover, the significant higher mean age at disease onset in group 1 ($P<0.0032$) is in accordance with some studies that have reported a more severe clinical course of MG in patients with disease onset after the fifth decade (152, 156, 157).

**Strengths of the study**

The present study has some important strengths. First of all is the large number of included patients. We included 232 patients, which represents a very large sample size considering the rarity of MG (2). The number of patients included in our study is also significantly higher than the 24 to 102 patients included in previous studies that investigated the prognostic value of SFEMG in MG (144-147). A large sample size is an important methodological strength, since it
increases the internal validity of the study by reducing the possibility of random errors in assessing a given exposure-disease relationship (158, 159).

Second, the internal validity of our study was additionally increased by controlling for confounders during data analysis (158, 159). The potential confounders that were considered were patients’ demographic characteristics (age and sex), AchR antibody status, presence of extra-ocular disease symptoms, presence of thymoma, thyroid pathology, other autoimmune, malignant or chronic diseases and thymectomy status. When we adjusted for the effect of these variables, the association of the initial SFEMG parameters with the disease outcome persisted, thus strengthening the validity of our results.

Third, we analyzed data obtained with SFEMG evaluation of the OOc. The later has a greater diagnostic yield in isolated ocular or mild generalized MG than the ED, used in other studies (138, 160).

**Limitations of the study**

Our study has also several limitations that need to be acknowledged.

First, due to the retrospective study design, we operated with a limited data set and we were not able to detect small changes in muscle weakness that eventually happened in individual patients during the follow up. For this reasons we could not use the detailed quantitative myasthenia gravis score (QMG (136)) as it is designed only for use in prospective studies. However, using the MGFA Clinical classification score, we were still able to detect major changes in muscle weakness that happened in association with severe disease exacerbations. Consequently, these limitations did not affect our ability to identify and correctly classify patients with severe disease exacerbations.
Second, since the patients were not treatment naïve throughout the entire study period, we could not definitely exclude the potential influence of treatment on the worst observed outcome. However, we can say that both outcome groups had the same treatment possibilities and were treated according to the severity of their disease exacerbations. Indeed, patients with severe disease exacerbation (group 1) were more likely to be treated with immunosuppression (table 8), perhaps due to more debilitating disease. In addition, the number of patients with severe disease exacerbations (group 1) represented 17% of all the patients in the study, and this percentage is in line with previously published data from observational studies on the frequency of severe disease course in MG patients, where the reported frequency was up to 20% (148).

Third, due to the retrospective study design, we determined SFEMG cut-off values for severe disease exacerbations using data obtained with a single fiber electrode (SFE). The SFE has been the standard electrode used for SFEMG (161) but is not going to be used anymore in the future due to changes in the hospital hygiene rules (162). The concentric needle electrode (CNE), which will replace the SFE, has a larger recording area with different reference values of MCD and jitter and potentially different cut-off values for prediction of the severity of future disease exacerbations (162, 163). However, since the difference of the reference values for MCD and jitter between the 2 types of electrodes is only minimal (the respective outlier limits for mean MCD and individual jitter are 20µs and 30µs for SFE and 27µs and 36µs for CNE), we speculate that the same is also true for the cut-off values that predict severe disease exacerbations. Consequently, the cut-off values of SFEMG parameters determined for the SFE in our study could possibly also be used for the CNE.
CONCLUSIONS

The results of the present study confirmed the research hypothesis.

In the study population the extent of the initial SFEMG abnormalities of the OOc (mean jitter value, percentages of motor end-plates with increased jitter and/or impulse blocking) correlated with the severity of the later clinical course of MG. Patients with a mean jitter value greater than 68.6μs, at least 81.3% of motor end-plates with increased jitter, and/or at least 21.8% of motor end-plates with conduction block developed severe disease exacerbations, whereas patients with lower mean jitter value, lower percentage of motor end-plates with increased jitter, and/or conduction block had a more benign clinical course.

FUTURE DIRECTIONS

The present study provides class II B evidence, that the extent of the initial SFEMG abnormalities in the OOc could be predictive of the severity of later clinical course of MG. Future prospective studies, performed with the use of a CNE, are required to confirm our results.
REFERENCES


11. Heldal AT. Myasthenia Gravis and Acetylcholine Receptor Antibodies. AChR-Antibodies as a Marker for Epidemiological Studies and in the Follow-up of Patients. Dissertation for the degree Philosophiae Doctor (PhD). University of Bergen, Norway; 2014.


164. **APPENDIX 1**: Certificate of study protocol approval by the National Medical Ethics Committee of Slovenia

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**KOMISIJA REPUBLIKE SLOVENIJE ZA MEDICINSKO ETIKO**

Mateja Baruca, dr. med.
Nevrološki oddelek
Slošna bolnišnica Izola
Polje 40, Izola

Štev.: 204/03/13
Datum: 10. 4. 2013

Spoštovana gospa dr. Baruca,

Komisij za medicinsko etiko (KME) ste 1. 3. 2013 poslali v oceno predlog raziskave z naslovom:

"Epidemiologija in zdravljenje miastenije gravis v Sloveniji."

Doktorsko delo Mateje Baruca, dr. med., pod mentorstvom doc. dr. Saše Šega Jazbec, dr. med.

KME je na seji 19. marca 2013 ocenila, da je raziskava etično sprejemljiva, in Vam s tem izdaja svoje soglasje.

Lep pozdrav,

prof. dr. Jože Trentelj
predsednik Komisije RS za medicinsko etiko

---

Naslov:
Prof. dr. Jože Trentelj, Institut za klinično neurofiziologijo, Univerza v Ljubljani, Založiska 7, 1025 Ljubljana, telefon 01/ 522 1425.
Tone Žarev, Universoterapii klinični center Ljubljana, Založiska 7, 1025 Ljubljana, telefon 01/ 522 1529.
APPENDIX 2: Original research paper

SINGLE FIBER EMG AS A PROGNOSTIC TOOL IN MYASTHENIA GRAVIS

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Accepted 2 May 2016

ABSTRACT: Introduction: Single fiber electromyography (SFEMG) is the most sensitive diagnostic tool for diagnosis of myasthenia gravis (MG). Its prognostic value is not known.

Methods: We retrospectively analyzed the clinical course of 232 MG patients who presented with only mild symptoms and had SFEMG of the orbicularis oculi muscle. We correlated their SFEMG results with the severity of their later clinical course.

Results: During the observation period 39 patients (17%) developed severe disease exacerbations, and 193 (83%) remained stable. Patients with severe disease exacerbation had a significantly higher mean jitter value (P < 0.0001), a greater percentage of fibers with increased jitter (P < 0.0001), and/or impulse blocking (P < 0.0001) on SFEMG. Conclusions: The extent of the SFEMG abnormalities in this study correlated with the later clinical course of MG.

Muscle Nerve 000:000–000, 2016

The clinical course of myasthenia gravis (MG) is variable, ranging from remission in an early stage to severe weakness and even death.1,2 Treatment strategies may differ for patients with severe disease; therefore, it is important to identify these patients as early as possible. At present, there is no known prognostic factor that would predict the long-term clinical course in MG patients at the time of diagnosis.

Single fiber electromyography (SFEMG) is considered the most sensitive diagnostic tool for diagnosis of MG.3,4 The sensitivity and specificity of the method depends on the muscle investigated and is the highest for the orbicularis oculi muscle (O Oc), with sensitivity of 94–99% and specificity of 85–98%.5 The typical SFEMG findings in MG are increased jitter and/or impulse blocking in some of the examined motor end-plates. In most patients with MG, disease severity correlates with SFEMG jitter and impulse blocking, but opinions differ regarding the prognostic value of the initial SFEMG data for the long-term clinical course. Some clinicians believe that MG patients with highly abnormal SFEMG results have a higher risk of developing severe disease, but many others doubt that this is a prognostic factor. However, objective evidence to support or refute this hypothesis is still lacking.

The aim of this study was to determine if the initial SFEMG values in the O Oc in MG are predictive of the severity of the long-term clinical course of the disease.

MATERIALS AND METHODS

Patients. This was an observational retrospective study on 232 patients with MG diagnosed in our departments between January 1, 2003, and December 31, 2012. The diagnosis of MG was based on the clinical picture of fluctuating muscle weakness and fatigability in association with increased jitter and/or impulse blocking on SFEMG and positive response to cholinesterase inhibitors and/or positive acetylcholine receptor (AChR) antibody assay. Only patients who presented with mild disease symptoms and had SFEMG of the O Oc at the time of diagnosis were included in the study.

Standard Protocol Approvals, Registrations, and Patient Consents. The study was conducted according to the Declaration of Helsinki and approved by the National Medical Ethics Committee of Slovenia. Informed consent was waived.

SFEMG. Jitter and impulse blocking were measured in the O Oc using the stimulated SFEMG technique.5 Stimulation was performed with a near-nerve needle in the facial branch to the muscle.

Jitter was expressed as the mean difference between consecutive action potential latencies (MCD) of 30 fibers. A study was considered abnormal if either of the following criteria were met: (1) the mean MCD (jitter) exceeded the upper limit of normal (ULN) for O Oc (MCD > 20 μs); or (2) more than 10% of individual fibers had an MCD greater than the ULN (50 μs for individual end-plates).6,7

AChR Autoantibody Assay. Serum immunoglobulin G anti-AChR antibodies were tested in all patients on samples collected at the time of diagnosis.
Autoantibodies were measured by radioimmuno-precipitation according to manufacturer’s instructions (IBL GmbH, Hamburg, Germany). A value of >0.4 nmol/L was considered positive.

Clinical Evaluation. We reviewed medical files of MG patients and collected initial SFEMG data, AChR antibody status, presence of thymoma, thyroid pathology, other autoimmune, malignant or chronic disease, thymectomy status, and use of immunosuppression. We correlated the percentages of increased jitter and conduction blocking with the patients’ later disease course.

Clinical Staging and Outcome Definition. The clinical stage was graded at the time of initial presentation and at the time of maximal worsening according to the Myasthenia Gravis Foundation of America (MGFA) Clinical classification score10 as follows: I, disease restricted to ocular muscles; II, mild weakness affecting limb, axial, or bulbar muscles, without interference with activities of daily living and responsive to cholinesterase inhibitors and/or low dose immunosuppressive drugs; III, moderate weakness which partially affects activities of daily living, responsive to immunosuppression with or without cholinesterase inhibitors; IV, severe weakness affecting limb, axial, or bulbar muscles which interferes with activities of daily living and requires treatment with plasma exchange or intravenous immunoglobulin; V, need for intubation, with or without mechanical ventilation. Only patients who presented with an initial MGFA clinical classification score of I or II were included in this study. The disease severity was then defined by the worst MGFA clinical classification score reached by the patient during the observation period.

According to the worst observed MGFA clinical classification score, the patients were divided into 2 outcome groups: Group 0, considered benign (patients who reached the worst MGFA clinical classification score of I–III), and Group 1, considered severe (patients who reached the worst MGFA clinical classification score of IV–V).

Statistical Analyses. Unless otherwise indicated, demographic characteristics are indicated by the mean ± standard deviation or median and interquartile range with respective 95% confidence intervals for continuous variables (for example, age). Proportions and 95% exact binomial based confidence intervals for proportions were estimated for categorical variables (for example, gender).

The difference between 2 groups for continuous variables was tested with \( t \) test, Welch \( t \) test, or Mann-Whitney test, where appropriate. The assumption of normality was verified with the Shapiro-Wilk test, and the Bartlett test was used to test the assumption of variance equality.

The association between 2 categorical variables (for example, gender and outcome group) was tested with the \( \chi^2 \) test with Yates continuity correction of the Fisher exact test, where appropriate.

The association between SFEMG parameters was estimated with Pearson correlation coefficients. Logistic regression was used to estimate the association between the SFEMG parameters and the outcome group. The Hosmer and Lemeshow test was used to test the goodness of fit of the model.

The cutoff values of SFEMG parameters were estimated by calculating the g-means (defined as geometric mean of sensitivity and specificity). Sensitivity and specificity were calculated with leave-one-out cross-validation from separate univariate logistic regression models.

A \( P \) value of <0.05 was considered to be statistically significant. The analysis was performed with R language for statistical computing (R version 3.0.1).11

RESULTS

The study group consisted of 232 patients with MG. At the time of diagnosis, when SFEMG was performed, all patients were in Class I or II of the MGFA Clinical Classification Score. During the observation period, 193 patients (83%) had a worst MGFA Clinical Classification Score of I–III and were thus classified into group 0, whereas 39 patients (17%) reached the worst MGFA Clinical Classification Score of IV–V and were, therefore, classified into group 1. The main clinical features of both groups of patients are outlined in Table 1.

Univariate analysis showed no statistically significant differences in gender frequencies, thymoma presence, thymectomy status, and median duration of follow-up between the 2 groups of patients. However, patients from group 1 (severe clinical course) were statistically significantly older (mean age at disease onset of 64.44 years), had a shorter time from symptom onset to SFEMG, were less likely to have isolated ocular disease symptoms, were more likely to be AChR-antibody positive, and were more likely to be treated with immunosuppression. Comparison for the presence of other comorbidities did not show any significant difference between the 2 groups of patients in terms of thyroid disease or other autoimmune, malignant, or chronic diseases. The respective percentages of affected patients in group 0 and group 1 with \( P \) values were 16.58% versus 20.51%; \( P = 0.7184 \) for thyroid disease, 9.33% versus 10.26%; \( P = 0.7708 \) for other autoimmune disease, 8.99% versus 10.26%; \( P = 0.7386 \) for other malignant and
Table 1. Clinical features of 232 patients with myasthenia gravis (MG) included in the study.

<table>
<thead>
<tr>
<th></th>
<th>Group 0 (worst MGFA Clinical Classification Score I-II)</th>
<th>Group 1 (worst MGFA Clinical Classification Score V-V-V)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n (%)</td>
<td>193 (83)</td>
<td>36 (17)</td>
<td></td>
</tr>
<tr>
<td>Subjects with ocular symptoms</td>
<td>122 (63)</td>
<td>16 (21)</td>
<td>0.0000</td>
</tr>
<tr>
<td>only at first presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(MGFA Clinical Classification Score I at first presentation, n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.8638</td>
</tr>
<tr>
<td>Woman (%)</td>
<td>104 (54)</td>
<td>21 (54)</td>
<td></td>
</tr>
<tr>
<td>Men (%)</td>
<td>89 (46)</td>
<td>16 (46)</td>
<td></td>
</tr>
<tr>
<td>Gender ratio, W:M</td>
<td>1:1.7</td>
<td>1:1.7</td>
<td></td>
</tr>
<tr>
<td>Mean age at disease onset ± SD</td>
<td>55.72 ± 17.81</td>
<td>64.44 ± 16.98</td>
<td>0.0002</td>
</tr>
<tr>
<td>Median time from symptom onset to SFEMG, IQR (months)</td>
<td>6; 1-12</td>
<td>2; 1-6</td>
<td>0.0150</td>
</tr>
<tr>
<td>AChR antibody status</td>
<td></td>
<td></td>
<td>0.0046</td>
</tr>
<tr>
<td>Positive (%)</td>
<td>111 (59.04)</td>
<td>33 (84.62)</td>
<td></td>
</tr>
<tr>
<td>Negative (%)</td>
<td>77 (40.96)</td>
<td>6 (15.38)</td>
<td></td>
</tr>
<tr>
<td>Thymoma</td>
<td></td>
<td></td>
<td>0.4571</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>10 (5.54)</td>
<td>3 (7.06)</td>
<td></td>
</tr>
<tr>
<td>No (%)</td>
<td>181 (94.46)</td>
<td>35 (92.94)</td>
<td></td>
</tr>
<tr>
<td>Thymectomy</td>
<td></td>
<td></td>
<td>0.9244</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>31 (16.32)</td>
<td>8 (15.38)</td>
<td></td>
</tr>
<tr>
<td>No (%)</td>
<td>159 (63.68)</td>
<td>33 (64.62)</td>
<td></td>
</tr>
<tr>
<td>Immunosuppression</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>70 (36.27)</td>
<td>35 (89.74)</td>
<td></td>
</tr>
<tr>
<td>No (%)</td>
<td>123 (63.73)</td>
<td>4 (10.26)</td>
<td></td>
</tr>
<tr>
<td>Median duration of follow-up time, IQR (months)</td>
<td>33.73; 13.7-68.27</td>
<td>34.33; 23.57-88.98</td>
<td>0.2402</td>
</tr>
</tbody>
</table>

Group 0, patients with benign clinical course (worst MGFA clinical classification score I-II); Group 1, patients with severe clinical course (worst MGFA clinical classification score V-V-V); MGFA, Myasthenia Gravis Foundation of America; SD, standard deviation; W, women; M, men; IQR, interquartile range; AChR, acetylcholine receptor.

57.51% versus 51.28%; P = 0.5900 for other chronic disorders.

There was a net difference between the 2 outcome groups when they were compared for SFEMG parameters. The main SFEMG characteristics for each outcome group are summarized in Table 2. Univariate analysis showed that patients from group 1 had a statistically significant higher mean jitter value and a greater degree of motor end-plates with increased jitter and/or impulse blocking.

The association between initial SFEMG characteristics and later clinical outcome was confirmed by logistic regression analysis. In the univariate models, higher mean jitter (OR = 1.0253; 95% CI: 1.0373-1.0347), higher percentage of increased jitter (OR = 1.0426; 95% CI: 1.0258-1.0597), and impulse blocking (OR = 1.0373; 95% CI: 1.0226-1.0521) on the individual motor end-plates correlated with a worse later MGFA clinical classification score (P < 0.0001).

For the multivariate logistic regression analysis, we made 3 multivariate logistic regression models considering separately each SFEMG parameter with other variables that were statistically significant in the univariate analysis. The reason for such

Table 2. SFEMG characteristics of 232 patients with MG.

<table>
<thead>
<tr>
<th></th>
<th>Group 0 (worst MGFA Clinical Classification Score I-II)</th>
<th>Group 1 (worst MGFA Clinical Classification Score V-V-V)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n (%)</td>
<td>193 (83)</td>
<td>36 (17)</td>
<td></td>
</tr>
<tr>
<td>Median jitter value, IQR (µs)</td>
<td>37; 26-66</td>
<td>96.5; 73-124.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median percentage of end-plates with increased jitter, IQR (%)</td>
<td>43; 18-83</td>
<td>94; 83-100</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median percentage of end-plates with impulse blocking, IQR (%)</td>
<td>10; 5-30</td>
<td>47.5; 23.5-60</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Group 0, patients with benign clinical course (worst MGFA clinical classification score I-II); Group 1, patients with severe clinical course (worst MGFA clinical classification score V-V-V); MGFA, Myasthenia Gravis Foundation of America; IQR, interquartile range.
an approach was a strong association between SFEMG parameters shown by the Pearson correlation coefficients (r = 0.799-0.872; P < 0.0001), due to which a single multivariate model could lead to invalid conclusions.

Multivariate logistic regression analysis showed that SFEMG parameters also remained significantly associated with the outcome when adjusting for the effect of other variables that were statistically significant in the univariate analysis. Higher mean jitter (P = 0.0025; odds ratio [OR] = 1.01630; 95% confidence interval [CI], 1.00566–1.02704), higher percentage of fibers with increased jitter (P = 0.0010; OR = 1.03190; 95% CI, 1.01271–1.05146), and/or impulse blocking (P = 0.0058; OR = 1.02977; 95% CI, 1.00687–1.04085) in individual motor end-plates were predictive of a severe clinical course.

Of the remaining variables that were significantly associated with the outcome in the univariate analysis, only higher patient age (P = 0.015–0.018; OR = 1.02905–1.03139; 95% CI, 1.00718–1.05511) and the presence of extraocular muscle weakness (P = 0.0005–0.0047; OR = 3.95292–5.02824; 95% CI, 1.52408–12.5489) were predictive of a severe clinical course, whereas AChR antibody status (P = 0.29395–0.65824) and duration of symptoms before diagnosis (P = 0.64622–0.82657) were not significantly associated with the outcome in the multivariate models. The more extensive use of immunosuppression therapy in the patients with severe disease exacerbations was considered a consequence of debilitating disease (note that all patients were treatment naïve at the time the SFEMG was performed). For that reason, the use of immunosuppression was not included in the multivariate logistic regression analysis.

The cutoff values of SFEMG parameters that predicted a severe clinical course were ≥68.61 μs for mean jitter, ≥81.31% of individual motor end-plates with increased jitter, and ≥21.86% of individual motor end-plates with impulse blocking, respectively (Fig. 1).

The respective cross-validated g-means (sensitivity, specificity) obtained with these cutoff values were 78% (77%, 79%), 77% (75%, 79%), and 74% (69%, 79%) for mean jitter, percentage of motor end-plates with increased jitter, and percentage of motor end-plates with impulse blocking, respectively. These values show good predictive ability of the SFEMG parameters.

DISCUSSION

We found that initial SFEMG of the OoC has prognostic value for the long-term clinical course of MG patients. In our study population, severe disease exacerbations were observed in subjects with a mean jitter value greater than 68.61 μs, at least 81.31% of motor end-plates with increased jitter, and/or at least 21.87% of motor end-plates with conduction block. On the other hand, patients with lower mean jitter value, lower percentage of motor end-plates with increased jitter, and/or conduction block had a more benign clinical course. This association persisted also when we adjusted for the effect of other clinical characteristics that could potentially influence the outcome according to multivariate logistic regression analysis (namely age and the presence of generalized disease symptoms).

Previous studies have shown that, in most MG patients, the change in SFEMG measurements correlated with the change in clinical state as measured by quantitative testing of muscle function.9-8 In these studies, the mean MCD increased by at least 19% in the tested muscle in two-thirds of the patients who become worse between consecutive
SFEMG examinations. Conversely, in over 80% of instances in which mean MCD fell by at least 10% between two SFEMG evaluations, there was definite clinical improvement.5-9 Meriggioli et al. confirmed the same decrease in MCD in patients with a positive response to immunosuppression therapy, indicating a potential value of SFEMG as a marker of early treatment response.10

To our knowledge, only 4 studies have investigated the prognostic value of SFEMG in MG patients.12-16 All of them addressed the ability of SFEMG of the extensor digitorum muscle (ED) to predict disease generalization in patients with ocular MG. Their results were conflicting. The first, a retrospective study on 24 patients, showed that disease generalization was more likely to occur in patients with mean jitter values exceeding 50 μs, whereas in patients with a lower mean jitter, the disease tended to remain confined to extraocular muscles.12 In the second, prospective study on 37 patients, a normal mean jitter was associated with MG remaining restricted to the extraocular muscles, but a higher mean jitter was not predictive of subsequent disease generalization.13 In the third and fourth retrospective studies made on 50 and 102 patients, respectively, SFEMG did not show any predictive value for later disease generalization in patients with isolated ocular disease.14,15 However, all of these studies were conceptually different from our study. The main differences were in the primary end-point and in the muscle used for SFEMG evaluation.

In contrast with previous studies, we used SFEMG to identify patients at risk for severe disease exacerbations. The choice of this primary end-point was based on data from historical observational studies, which showed that up to 20% of patients with MG experience severe disease exacerbation during their lifetime.16 Identification of this subgroup at the time of diagnosis would allow for more intensive follow-up and early initiation of effective immunosuppressive treatment. Accordingly, severe, potentially life-threatening disease exacerbation could possibly be prevented.

The choice of the muscle for SFEMG was a direct consequence of the differences in the primary end-point and tested hypothesis. In all previous studies, the main idea was that there is a qualitative difference in electrodiagnostic muscle involvement between patients with ocular MG destined to remain ocular and patients who later generalized.12-15 In contrast with patients destined to stay ocular, patients who later developed generalized disease were suspected to have subclinical electrodiagnostic involvement of other skeletal muscles. For this reason, SFEMG was performed on the ED, which was believed to have increased mean jitter values only in patients who would later experience disease generalization. However, this supposition failed to be confirmed.

In our study, we tested a different hypothesis. Studies of normal and abnormal neuromuscular transmission with SFEMG showed that the extraocular muscles, and in particular the OOc, are electrodagnostically affected in virtually all MG patients.7 However, despite being affected, the extraocular muscles are not always symptomatically weak.17 The susceptibility of extraocular muscles for MG pathology is probably the result of differences in neuromuscular junction morphology and physiology (they have less prominent synaptic folds, fewer postsynaptic AChRs, and smaller motor units that are subjected to high firing frequencies); in addition, they have a low expression of complement regulators that might make them more vulnerable to complement-mediated damage.17,20 In this context, the extent of changes in jitter could function as a surrogate marker of the autoimmune process at the level of the neuromuscular junction. Our main idea was that a higher mean jitter value in the extraocular muscles could be potentially predictive of later severe disease exacerbations. Hence, we chose the OOc for SFEMG evaluation.

Another aspect of our data that deserves a special comment is the age of the patient population at disease onset. Because the mean age at disease onset was relatively high in both patient groups (55.7 years in group 0 and 64.4 years in group 1), it would seem that our patient population is too old to function as a model for long-term disease outcome. Nonetheless, in most MG patients the disease course is determined early (77-82% of patients reach maximum weakness in the first 2-3 years).21,22 In addition, several previous and recently published review articles and epidemiological studies have reported a continuously increasing incidence of MG in patients above age 50 years.23-26 Therefore, the relatively high mean age at disease onset of our patient population should be considered both a representative sample and a consequence of the current epidemiology of MG. Moreover, the significant higher mean age at disease onset in group 1 (P < 0.0032) is in accordance with some studies that have reported a more severe clinical course of MG in patients with disease onset after the fifth decade.22,27,28

The major strengths of this study are the large number of included patients, the considerations of the influence of other patient clinical characteristics on the outcome, and the use of the OOc for SFEMG. The latter has a greater diagnostic yield in MG than the ED, used in other studies.
However, our study has also several limitations that need to be acknowledged. First, due to the retrospective study design, we were not able to detect small changes in muscular weakness. For this reason, we could not use the detailed quantitative myasthenia gravis score as it is designed only for use in prospective studies. However, using the MGFA Clinical Classification score, we were still able to detect major changes in muscular weakness that happened in association with severe disease exacerbations. Consequently, these limitations did not affect our ability to identify and correctly classify patients with severe disease exacerbations.

Second, because the patients were not treatment-naïve throughout the entire study period, we could not definitely exclude the potential influence of treatment on the worst observed outcome. However, we can say that both outcome groups had the same treatment possibilities and were treated according to the severity of their disease exacerbations. Indeed, patients with severe disease exacerbation (group 1) were more likely to be treated with immunosuppression (Table 1), perhaps due to more debilitating disease. In addition, the number of patients with severe disease exacerbations (group 1) represented 17% of all the patients in the study, and this percentage is in line with previously published data from observational studies on the frequency of severe disease course in MG patients, where the reported frequency was up to 20%.

Third, due to the retrospective study design, we determined SFEMG cutoff values for severe disease exacerbations using data obtained with a single fiber electrode (SFE). The SFE has been the standard electrode used for SFEMG but is not going to be used anymore in the future due to changes in the hospital hygiene rules. The concentric needle electrode (CNE), which will replace the SFE, has a larger recording area with different reference values of MCD and jitter and potentially different cutoff values for prediction of the severity of future disease exacerbations. However, because the difference of the reference values for MCD and jitter between the 2 types of electrodes is only minimal (the respective outlier limits for mean MCD and individual jitter are 30 µs and 90 µs for SFE and 27 µs and 36 µs for CNE), we speculate that the same is also true for the cutoff values that predict severe disease exacerbations. Consequently, the cutoff values of SFEMG parameters determined for the SFE in our study could possibly also be used for the CNE.

In conclusion, this study has shown that the extent of the initial SFEMG abnormalities in the OOC could be predictive of the severity of later clinical course of MG. Future prospective studies, performed with the use of a CNE, are required to confirm our results.

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REFERENCES